

More wicked ways with hormones

The European Commission has hung an intellectual millstone around its neck by banning the use of bovine growth hormone in growing cattle. The European Parliament has a duty to put it straight.

VISIONARIES who proclaim that the United States of Europe is no further away than 1992, when the European Economic Community will constitute the single market originally decreed by the Treaty of Rome, habitually overlook the still-glaring differences between Europe and the United States. True, Europe has a president (now M. Jacques Delors), but does not require that he should be elected as Mr George Bush has been. And while the members of the European Commission (now 17 strong) are roughly analogous to members of a cabinet, they too are merely nominated every four years by the political heads of member governments. There is also a single-chamber European Parliament, the equivalent of the US Congress, which does suffer periodic re-election (next in June this year), but the opinion even among ardent Europeans is that the parliament will have to win influence in European affairs by demonstrating its value and good sense. The same Europeans will therefore be dismayed by one development last week — the decision of a parliamentary committee to support and even to reinforce the regulations that prohibit the use of bovine growth hormones in the fattening of cattle.

The circumstances are quite scandalous. Since the beginning of 1988, the sale in Europe of beef grown with the use of bovine growth hormone has been prohibited by a directive (with the force of law) of the European Commission. Although the measure has been justified as a means of protecting people's health, there is literally no evidence that beef produced with growth hormone is a hazard to health, but every reason to believe that bovine growth hormone in cattle is rapidly metabolized (which is why it helps cattle to grow more quickly). So why ban its use? Because Europe's system of support for agriculture is a means of over-producing all kinds of commodities, beef included, and because those administering the system were ready to clutch at any straw to prevent further surpluses of beef. What more natural than an alliance with the green extremists to keep the hormone out of use? Although imports of beef from the United States were exempted from restriction during 1988, this year began with an acrimonious trade dispute between Europe and the United States that rumbles on.

Now the European Parliament has entered the fray, but discredibly. Last week its environment committee in Luxembourg issued the gist of a report supporting the European Commission's ban and urging more vigilance in

tracking down now-illicit uses of these materials. The opportunity for extricating the Commission from the pit of unreason it had dug for itself has been spurned in the face of informed opinion and despite the diplomatic damage the ban has already caused. In the short run, the consequence will be more trouble. Unless the full parliament rejects the opinion of its committee in the discussion arranged for April, it will find that in the long run, it has much less influence than it might have won by acting sensibly, which should worry ardent Europeans, green and not-so-green. □

Budgets off balance

Budget problems in Britain, the Soviet Union and United States may have different origins — but one solution.

AMONG the quaintest British customs is the annual ritual in which a person called the Chancellor of the Exchequer (now Mr Nigel Lawson) tells the House of Commons what taxes he plans to increase or decrease. The procedure is the inverse of what happens in most other places, where a government's annual budget is a statement of what it plans to spend coupled with a comparison of total spending and total revenue. In Britain, the spending estimates appear first, as fiat. That is why Lawson's performance last week (his sixth) has generally been written off as "dull" for, breaking with recent form, he offered neither tax breaks nor further imposts, except marginally.

But there is more than that to say. The British government is in the enviable position that its accounts are in surplus. In the year ending this month, there will be a surplus of £14,000 million. With the over-cautious forecast that next year's surplus will be the same, the British government could liquidate its entire debt within a decade. But is there nothing better to do with surplus funds than pay off debts? Not on British experience in the past few years. Putting more money in British pockets (either by reducing taxes or spending money) seems either to increase imports or industrial costs (through higher wages) — or both. Britain already has a trade deficit comparable with its budget surplus and slightly larger (as a percentage of Gross Domestic Product) than that of the United States. And inflation is pushing 8 per cent, so that interest rates have increased and sterling has



been allowed to increase in value against other European currencies. Lawson made a clean breast of his dilemma last week: despite the £14,000 surplus, he had no room for manoeuvre.

Notoriously, exactly the same is true in the United States, where the Deficit Reduction Act (better known as Gramm–Rudman after two of its three sponsors) puts a statutory and reducing ceiling (to be fixed finally only in the summer) on the federal budget deficit. Again, the government has no room for manoeuvre. Part of the trouble is that more than a third of US spending is fixed by various acts of Congress, while just under a third goes on defence. Because savings in the United States are not great enough to bridge the gap, the deficit has been financed by borrowing from elsewhere (which is why there is a trade deficit). Over the past 18 months, two devices have been used to correct the balance — interest rates have been increased (as in Britain) but the value of the US dollar has been allowed to decline. This house of cards depends on people's willingness to continue turning foreign currencies into dollars to bridge the gap; ideally, the lenders would have been given proof of a deal between the administration and the Congress early in the new presidency, but even optimists are now talking of cutting a deal only in the summer. The sharp if modest fall of the stock markets last week, ostensibly from fears of still higher interest rates, may be a sign that the delicate apparatus has begun to crumble.

Mr Mikhail Gorbachev is similarly hemmed in. The Soviet Union's budget is believed to be 20 per cent in deficit, but the consequent inflation is only marginally apparent because most prices are centrally controlled. Because there is so little on which people can spend money, they save it against the time when the shops are full again. Last week, Gorbachev outlined an agricultural reform that would have seemed revolutionary before he came to office; farmers are to be allowed personally to lease the land they farm on the grounds that they will then become more efficient, but the details are not yet made public and are probably still to be decided. One snag, not nearly as well advertised, is that even more efficient farmers will not grow more food unless prices are also increased, perhaps even by a free-market mechanism. Sadly, even the bare bones of the scheme will give ideological offence.

Why should three substantial governments be thus hamstrung? To compare the United States and the Soviet Union economically is absurd, but that is not the end of it. The United States is locked into a straitjacket because, despite the astonishing flexibility of its economy and the steady growth of production, expectations have stolen a march on real wealth. President Bush's hope, like his predecessor's, is that the United States will grow out of trouble, but even the present modest rate of growth is judged dangerously fast. The safer short-term remedy is modestly to reduce consumption, which the new president has promised he will not, but in the long run only an increase of productivity (rather than of mere production)

will square the circle. Exactly the same applies in the Soviet Union, but should be more achievable, given both the encouraging and the admonitory examples with which the world is littered. And, as it happens, the same long-term remedy is what Britain should also be looking for. The box in which Lawson is imprisoned would be less constraining if it were possible to spend (or give away) some or all of that budget surplus without rocking the boat. Maybe the three people chiefly concerned should put their heads together. □

Universities in arms

British academics are faced with a nasty choice between a poor pay award and a continuing labour dispute.

THE reasons for believing that British academics are underpaid do not strain the imagination. Salaries have grown less quickly than those in other professional walks of life over the past decade of general deprivation for universities, and now compare so much less well than those in industry and elsewhere that university teachers and researchers are on the march to other jobs. The more serious worry is that recruitment to academic research at all levels is quickly drying up. Even school-leavers have learned that the academic profession is not much valued by the academic system's paymasters. Since the beginning of this year, academics have been declining to mark examination papers; now they have to choose between the continuation of that fruitless pursuit and their employers' offer of a salary increase of 6.5 per cent (which for practical purposes covers a two-year spell). The best course is to settle for what is on offer, but to prepare to fight a better battle next year.

The world, by now, knows that the vast majority of universities do not have the funds with which to pay more than they have offered. The whole world should also know that the British system cannot continue as in the past without running into serious difficulties. Hitherto, British academic pay has been determined nationally, by negotiation between the Association of University Teachers and the Committee of Vice-Chancellors and Principals. But the British university system is now well on the way to a welcome diversity in which some universities will be more able than others to pay good salaries. Disparities can only be further accentuated if the British government decides that the time has come to increase, perhaps double, the tuition fees paid from public funds to the universities which students attend, but recovering the cost from running budgets. Then universities will be competing tooth and nail for students.

Whether academics (or their union) welcome this development, it is happening and will continue. Nationally negotiated pay awards are bound eventually to disappear. It would be sensible to anticipate that certainty, if only for the sake of the greater say that academics would thereby acquire in the management of their own institutions. □

Soviet Academy faces grass-roots revolt

- Academy's official candidates at risk
- Democratic fervour takes a hold

Moscow

THE nomination conflict at the Academy of Sciences of the USSR is building up to a dramatic denouement this week — the academy's list of candidates may even be voted down by the academy's research institutes. The conference at which the academy will elect 20 of the 23 nominated candidates was scheduled for 20–22 March, at the Youth Cultural Centre in Moscow. The voters will be the 907 full and corresponding members of the academy and 440 institute representatives.

Since the January meeting at which Academicians Andrei Sakharov and Roald Sagdeev were voted down despite the recommendation of many institutes, ordinary scientists have seen the outcome as a blatant disregard of their opinions. After the protest meeting outside the academy on 2 February (see *Nature* 337, 593; 1989), several institutes formed a group to press for democratic elections.

Now the group has held a meeting of Moscow-region delegates to this week's electoral conference at which it was agreed to vote against all 23 official nominees unless the list is abrogated or enlarged (but enlargement is no longer possible). The Moscow region accounts for nearly half of all academy scientists.

The meeting emphasised that its intended "No" vote is not directed against particular nominees but is a protest against the "list imposed on the academy conference despite public opinion".

Even so, the outcome may be dramatic. Under the election rules, candidates who

fail to secure at least one vote more than half of the total eligible to vote will not be elected. If the number of successful candidates is fewer than 20, the conference will have to nominate further candidates on the spot, which is what the protesters want. Much will depend on the full and corresponding members of the academy, who will have a majority this week.

Will the academy bosses allow those with fewer letters after their names to carry the day? The answer cannot be simple. Not only the election is at stake, but the other issues triggered by January's nomination meeting, which in retrospect is the stone that started an avalanche of reforming public opinion in science.

Democratic reform is sweeping the academy. The procedure of electing institute, department and laboratory heads has been changed beyond recognition. Research groups compete for research grants, which is a novel development. Think tanks are set up for particular problems in research and development.

But the outdated is stubborn and tenacious, even though the backward-looking do not offer open resistance. Moth-eaten patterns of behaviour have inertia, and the research community is wary of risky ventures.

The protest meeting at which the decision was taken to vote "No" against the official list of candidates was also the first to voice a now-popular idea — that of creating an independent Soviet scientists' union. The praesidium of the academy supported this initiative at its meeting of 7 March, and appointed a working-group under Yuri Osipyan, an academy vice-president and director of the Moscow Solid State Physics Institute, to study the idea; a national researchers' conference is planned.

But the inter-institute activist group is in a ferment, regarding the academy's action as a means of appropriating its own project and of attaching the budding union to the praesidium.

At a rally of Moscow researchers which I attended on 11 March, the union's prospects were debated. Although the organizers had invited members of the academy's upper echelons, the bosses were conspicuous by their absence. The rally adopted the text of an address to Soviet scientists about the formation of the union, and elected an organizing committee with close on 40 members. An inaugural conference is expected in May.

Yuri Kanin

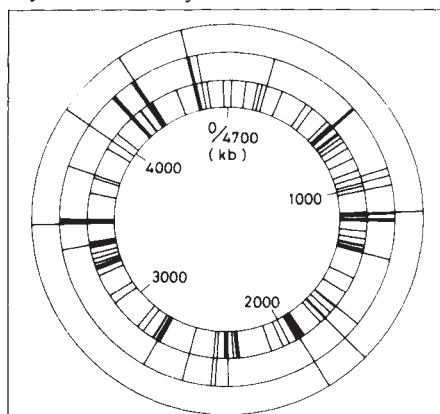
Nature Moscow correspondent/Novosti

Full sequence for *E. coli*

Tokyo

JAPAN'S Ministry of Education, Culture and Science (MESC) will shortly announce its backing for a project to sequence the entire genome of the bacterium *Escherichia coli*. With luck, *E. coli*'s estimated 4,700 kilobase pairs, one thousandth the number of the human genome, could be sequenced in five years. Funding for the project will remain uncertain until the Diet passes the 1989 budget, delayed by political quarrels over the Recruit bribery scandal. But given the scale of MESC's 'priority areas of research' fund, it seems likely that more than a million dollars a year will be provided for an initial three years.

The project is led by Takashi Yura of Kyoto University and Katsumi Isono of



By July 1987, most of the *E. coli* genome had been cloned, as shown in the outer ring here. The bars represent uncloned regions and the inner two rings show the 'gaps' closed by successive clonings. Scale in kilobases from 0 min. the *thr* locus. (Modified from Y. Kohara, K. Akiyama and K. Isono, *Cell* 50, 495; 1987.)

Kobe University and builds on a physical map of the *E. coli* chromosome put together from 3,400 clones (see box). Researchers from Kyoto, Kobe, Osaka and Tokyo universities will make up the core of the project, but international collaboration should become possible once a clone bank has been established at the National Institute of Genetics at Mishima.

Of course, *E. coli* has already been extensively studied. More than 1,000 genes have been mapped and about 450 kilobase pairs have been sequenced by researchers around the world. Isono says he hopes his laboratory alone will be able to manage 200–500 kilobases a year. He adds a note of caution, however, because in sequencing projects, "90 per cent of the work is done in 50 per cent of the time", with unbridgeable gaps remaining.

If successful, the complete *E. coli* sequence will be the first for an independently living organism. **Alun Anderson**

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Moscow spring: thousands marched in Moscow in support of pro-perestroika Boris Yeltsin in Sunday's election.

California may take hard line

Berkeley

THE University of California (UC) is contemplating changes to its tenure policy for faculty members that would permit the removal of the "grossly incompetent". The new policy was approved by the Berkeley faculty senate last month; other campuses are considering adopting the same rules. Supporters of the new measure say it provides clear and specific criteria by which to judge faculty performance, specifying as it does dismissal rather than demotion for those judged incompetent.

US universities have been forced to tackle the issue of faculty competence, especially where older people are concerned, because federal law may soon prohibit compulsory retirement ages. Indeed, universities were exempted from the federal Age Discrimination in Employment Act of 1984, which made mandatory retirement illegal, and allowed to require retirement at 70, but that exemption will lapse in 1993 if not renewed by Congress.

Out of a general concern over "non-productive faculty", UC began in 1984 to review all tenured faculty members who had not appeared before a promotions or other committee for at least five years. During the first round of reviews, five people were found to be both creatively unproductive and grossly deficient in teaching.

While university rules allow that tenure may be terminated "for good cause", the



statement is so vague as to be worthless, according to UC Berkeley engineering professor Ernest Kuh, chairman of the committee responsible for the new proposals. But the new policy, he says, breaks new ground, and other universities may wish to follow.

Under the new proposals, a faculty member, to be judged incompetent, must have both "ceased to engage in serious scholarship or creative activity for a substantial period of time" and be teaching in a manner "so inadequate that it is a dis-

service to students". Faculty members whose five-year evaluations produced such a record would be offered counselling and assistance, such as retraining, says English professor Ralph Rader, who presented the proposal to the academic senate last month. Only if those failed would dismissal be considered.

The purpose of the proposal is to set up machinery for dealing with incompetence that is so formalized and specific, says Rader, that it will automatically be set in motion by a negative review. But he expects that most such findings would lead not to unconditional dismissal, but to negotiated early retirement.

INTERNATIONAL RELATIONS

Sierra Leone takes on the Soviet Union

London

THE Soviet Union is considering suing the Sierra Leone government for damages following the recent 8-day detention of the research vessel *Akademik Mstyslav Keldysh* in the port city of Freetown.

The peculiar incident began last month when the oceanography research vessel, owned by the Academy of Sciences of the USSR, arrived in Freetown to pick up some representatives of a West German company that had recently completed repairs to the two deep-water submersibles carried on the ship. The plan was to test the repaired submersibles at sea 100 km west of Sierra Leone, where conditions are favourable at this time of year.

But when the ship docked at Freetown, it was boarded by Sierra Leone officials who removed the captain and several others, and questioned them in a local hotel. When the West Germans arrived in Freetown by air, they too were detained and questioned.

The Soviet foreign ministry immediately issued a strongly worded protest, calling the detention of the ship "a case of deliberate provocation". Soviet officials maintain that the Sierra Leone government was given 72 hours' notice of the ship's intention to dock at Freetown. Other countries, including Britain, whose scientists were also aboard the vessel, seemed less inclined to become involved in the dispute.

The Sierra Leone government has not explained why the ship was detained. According to the ship's captain, the Sierra Leoneans evidently believed either that the ship was on a military mission, or that it was intending to dump toxic waste within Sierra Leone's territorial waters.

The ship was eventually permitted to leave, but the normally cordial diplomatic relations between the two countries are now strained.

Vera Rich

None of the five faculty members at Berkeley judged incompetent was close to retirement age, says Rader, who emphasizes that the policy is not age discriminatory. "The aim", he says, "is to deal with incompetence without reference to age."

Elizabeth Fader, of the American Association of Universities, says that concern over incompetence is rising at many universities, although most of the proposals for dealing with it have focused on incentives for early retirement, aimed at offsetting the ending of compulsory retirement ages. The National Academy of Sciences has begun a study, expected to be completed in two years, of the potential consequences for universities of the elimination of compulsory retirement.

Marcia Barinaga

SOUTH AFRICA

Kane-Berman wins reinstatement

Cape Town

DR Jocelyn Kane-Berman, who was dismissed as superintendent of Groote Schuur Hospital in November after naming Nelson Mandela as her choice of premier in a newspaper interview (see *Nature* 338, 4; 1989), has been reinstated in her post with immediate effect.

The Administrator of the Cape Province, Mr Gene Louw (under whose aegis hospital administration falls) said on 10 March: "I can merely say that I took the initiative to bring it about. I thought it was in the best interests of Groote Schuur Hospital." This complete change in the government's attitude seems to be related to an appeal by Kane-Berman to have her reinstatement ordered by the courts. She had already served papers on Louw and other provincial officials, and her case was due to be heard in the Cape Supreme Court on 20 April. In December, Louw described Kane-Berman's removal as "final from the outset", and last month in parliament the Minister of Health and Population Development, Dr Willie van Niekerk, refused to intervene.

Kane-Berman has now agreed to withdraw the legal proceedings, and agreement has been reached on costs. She has, however, retracted her comments in the weekend newspaper in which she made her "controversial" statement, and has tendered her apology towards those persons and offices that were embarrassed. It is rumoured that South Africa's ailing State President, P.W. Botha personally ordered Kane-Berman's removal, although this has been denied by government spokesmen.

Kane-Berman, who described herself as "quite delighted" by her reinstatement, said: "I'm very pleased that the matter has been amicably resolved." Michael Cherry

Kansai science city takes root

Kyoto

WITH the opening last week of a new advanced telecommunications research institute, the Kansai region of Japan took its first step towards building a massive \$25,000 million 'Science City' designed to challenge the Tokyo region's dominance in high technology.

The Kansai region, encompassing Kyoto, Osaka and Kobe, is a powerful force in the world economy. Its gross 'national' product is twice that of Australia or the Netherlands, and by 1992 it will have Japan's first 24-hour international airport, providing direct access to foreign capitals.

Tax concessions will encourage research and educational institutes to move to the city. But the intention is to avoid the mistakes made in Japan's last giant science city project at Tsukuba, an hour's ride from Tokyo. Tsukuba was created by re-locating 46 government research institutes and two universities from cramped quarters in Tokyo to a undeveloped site. But the city's cultural amenities never caught up with its laboratories and many of its research personnel prefer to commute from Tokyo than to live among the paddy fields.

Kansai's science city is planning for slower and more dispersed growth that will integrate it into surrounding communities. And rather than government laboratories, its focus will be on the development of "the basic sciences and their combination with applied technology". To help towards this objective, an International Institute of Advanced Studies, modelled on those at Princeton and Aspen in the United States, and a new national library, to rival that at the Diet in Tokyo, were planned as early components of the city. The institute will be built alongside

Advanced Telecommunications Research Institute International (better known as just ATR), the first major new laboratory at the city site.

ATR, backed by private industry, the Ministry of International Trade and Industry (MITI) and the Ministry of Posts and Telecommunications, sees little challenge left in developing telecommunications to move information economically from one fixed location to another. Rather, the institute looks to the long-term basic research that will provide automatic translation telephones, totally portable communications systems making it possible to call "anyone, anywhere, at any time", and neural-network computers to provide friendly man/machine interfaces. The institute has begun work with 160 researchers and is expected to have 300 by 1995.

Two government-supported projects are likely to come to the city: a MITI Institute for Ion Engineering, and a Ministry of Education, Culture and Science (MESC) graduate study institute. The MESC institute will be Japan's second 'graduate university', designed to boost the comparatively low number of postgraduate students in Japan, provide graduate students for the research institutes which are not attached to universities and exercise MESC's newly approved right to accept industrial funding for university chairs.

Private-sector companies will provide the great mass of the research institutes. Land has already been set aside for 10 such institutes. Biotechnology may turn out to be a strong theme, given that more than half of all Japan's major biotechnology and pharmaceutical companies are based in Kansai.

Alun Anderson

MICROCHIPS

Britain bows out of hi-tech

London

THE sale last week of Britain's microchip company, Inmos, best-known for its revolutionary transputer, to SGS-Thomson (ST), a semiconductor manufacturer formed by the Italian and French governments in 1987, could mean that Europe has a semiconductor group capable of competing with the world-leaders from Japan and the United States. But the loss of British control illustrates the unwillingness of the country's industry and government to take a longterm view of investment in technology.

Britain's only other microchip manufacturer, Plessey, is also likely to go from national control into the hands of the West German company Siemens and the British company GEC; the takeover is under review by the Monopolies and Mergers

Commission. The British government, after launching Inmos in 1978, sold it six years later, before it made a profit, to the entertainments group Thorn EMI. But Inmos was seen as a high risk for the lighting and television rental group, making profits last year for only the first time since the beginning of the 1980s.

There were fears of redundancies in the three main Inmos plants, which employ over 1000 people, but a spokesman for the company says that ST plans to increase investment in the company, making Britain the headquarters of its microprocessing capability and possibly creating more jobs. For the sale of Inmos, Thorn EMI receives a 10 per cent shareholding in ST, and will continue to obtain the revenue from some patents on Inmos technology.

Christine McGourty

Technology transfer

THE transfer of technology from universities and national laboratories into industry receives less government support in Britain than in West Germany, France and Japan, despite the relatively low industrial investment in research and development in Britain which makes technology transfer more important, according to a report published last week by Britain's National Economic Development Council. The report compares efforts at technology transfer in Britain, the United States, Japan, France and West Germany. Britain's efforts compare badly with those of its competitors. The report says that British companies are reluctant to take up external technology and do not view technology as a long-term asset.

C.McG.

Radioactive waste

THE choice of sites for a deep repository for low-level and intermediate-level radioactive waste in Britain has been narrowed down to two: Sellafield in Cumbria and Dounreay in Caithness. Both are already the sites of nuclear installations. After permission is obtained from the planning authorities, geological investigations will determine which site is chosen. United Kingdom Nirex Limited, which is responsible for the disposal of low- and intermediate-level radioactive waste says each repository could cost about £3,000 million and should be in operation by 2005.

C.McG.

Language schism

ANATOL Sarokin, a geologist with the Byelorussian Hydrogeological Survey, has been threatened with dismissal for insisting on his right to use the Byelorussian language for his field notes and reports. His superiors say that only Russian may be used at work, but Sarokin maintains that as it is a Byelorussian survey, reports should be in Byelorussian — particularly at a time when so much emphasis is being publicly put on the fact that the Byelorussian Republic is 'bilingual'. But the directors of the survey say that reports must be in Russian because a copy has to be sent to Moscow, and they cannot afford to have them translated. Sarokin's case has been taken up by the weekly *Litaeratura i Mastastva*, the main advocate of *perestroika* in the Byelorussian Republic.

V.R.

Greenpeace recognized

THE Baltic Marine Environmental Protection Commission, established in 1980 by the governments of the seven littoral states (Denmark, Sweden, Finland, the Soviet Union, Poland and East Germany and West Germany) has changed its statutes to give observer status to the non-governmental group Greenpeace. At its regular meeting in February, the commission also resolved to set up an early-warning system for incipient environmental disasters; each participating state will warn the others if it observes unusual and disquieting phenomena in the marine environment.

V.R.

Canada's NASA

CANADA finally has a space agency. After months of squabbling over where it should be located, the government announced earlier this month that the new agency's headquarters would be in the greater Montreal area. Until now, responsibility for Canada's space activities fell to many ministries. The decision to base the new agency in the province of Quebec ends a political battle between predominantly French-speaking Quebec and the predominantly English-speaking Ontario that held up plans to establish the agency. In addition to coordinating Canadian activities on the space station, the agency will manage RADARSAT, a planned Earth remote-sensing satellite, MSAT, an advanced communications satellite, and the Canadian astronaut office. The current fiscal year's space budget is C\$120 million, rising to C\$150 million next year.

Larkin Kerwin has resigned as president of the Canadian National Research Council to become president-designate of the new agency. J.P.

President removed

DR Pulat Khabibullaev, nuclear physicist and former president of the Uzbek Academy of Sciences, has been replaced as president of the Uzbek Republic on the grounds of corruption. He was accused of having "actively assisted" the scientific careers of close relatives of now-disgraced local party leaders, procuring them posts for which they were insufficiently qualified and which they treated as virtual sinecures. Furthermore, he is said to have used his position as vice-president and then president of the academy to have his name attached as co-author to no fewer than 322 scientific papers to which he had contributed nothing. Called to account before the bureau of the Central Committee of the Communist Party of Uzbekistan, Khabibullaev made a formal self-criticism, whereupon the Party recommended that he be allowed to resign as president of Uzbekistan and "transferred to scientific work". V.R.

Fang speaks out

DR Fang Lizhi, the Chinese astrophysicist and dissident who was prevented by the police from attending US President George Bush's banquet in Beijing, has not let the incident pass unchallenged. At a press conference at his home the following day, he suggested that the reluctance of the Chinese leadership to have dinner with one dissident on one single occasion must surely raise doubts about their "promised tolerance" of the future presence of non-socialist Hong Kong in post-1997 China. Official Chinese spokespersons continue to attack the original invitation as "disrespectful" to the president's official hosts — the Chinese leaders — and US reaction to Fang's exclusion as "irresponsible". V.R.

Outside influences resented

São Paulo

SOUTH American government officials promised at a series of meetings last week to respect the rain-forest environment while promoting development, and called for more research on the damage to rain forests. But a concurrent scientific meeting on rain-forest ecology dismissed these promises as mere rhetoric, and complained that researchers are being denied resources.

The foreign ministers of Ecuador, Brazil, Colombia, Venezuela, Peru, Surinam, Guiana and Bolivia gathered in Quito, Ecuador, last week for the third meeting of the Amazonian Cooperation Treaty, signed in 1978 to defend the region's environment. The ministers favour "rational" use of Amazonian resources, and condemn "any foreign interference on the policies and actions that the Amazonian countries make in the region".

The governors of several Brazilian states were in Manaus to discuss the newest federal environmental protection programme called "Nossa Natureza" ("Our Nature"). Announced last year as a response to growing internal and international criticism of rain-forest devastation, the programme consists of legislation strengthening forestry and mining codes and creating new protected areas. Brazilian President Jose Sarney is due to sign these measures on 6 April.

But scientists from INPA — Instituto Nacional de Pesquisas da Amazonia (National Institute of Amazon Research) — think otherwise. They feel left out of the planning of "Our Nature" and fear that it is going to be just another meaningless declaration of good intentions. Researchers from the institute held a last-minute "alternative" meeting on 7 and 8 March in Manaus to draft a response to the "Our Nature" approach, but were prevented from presenting their conclusions to the governors meeting nearby.

Brazilian Minister for Industrial Development, Science and Technology, Roberto Cardoso Alves, said in February that the rain forest is "practically intact", with just 1 per cent destroyed. Estimates from INPA put the figure at 8 per cent.

If governments are resisting foreign intervention, the same cannot be said for researchers. INPA has many foreign scientists, mainly US and German. One US scientist, Philip M. Fearnside, has become the institute's media star, by his constant advocacy of preservationist measures. He complains that red tape is drawing away many researchers from the Brazilian side of Amazonia: there are stories that some foreign scientists have had to wait a year for a visa to visit Brazil.

INPA researcher Muriel Saragoussi says the institute may lose more than a

third of its 284 researchers if a government decision to dismiss all public servants with less than five years on the job takes effect. The proposed layoffs are a result of a January economic plan by the federal government to try to stave off hyperinflation. Another consequence of this plan is a reduction of 50 per cent in the financing of research institutes.

Research on rain forests will certainly be an important part of the planned International Geosphere-Biosphere Programme (IGBP). The programme's executive director, Thomas Rosswall, toured last week through several Latin American countries to promote the programme. He diplomatically stated in São Paulo that Brazil has sovereignty to decide what to do with its natural resources, but added that all countries will have to take responsibility for what happens to the world's climate. He compared the situation of the rain forest with the emission of sulphur dioxide by Britain; only when scientists showed that British emissions were polluting Europe did the British government take measures to curb them. Brazil may face similar pressure when the true importance of the rain forest to global climate change is conclusively shown. IGBP is likely to place one of its proposed global observatories in the Latin American rain forest. **Ricardo Bonalume Neto**

Mercurial approach

São Paulo

THE Brazilian government has been somewhat mercurial in its approach to science and science policy. Two months after deciding to abolish the Ministry for Science and Technology, it announced last week that it was creating a new, cabinet-level Special Secretary for Science and Technology. The two-month-old Ministry of Industrial Development, Science and Technology, which emerged in the wake of the departure of the four-year-old science ministry, now becomes the Ministry for the Development of Industry and Commerce.

Critics say this confusion of names and ministries underlines the lack of a coherent policy for Brazil's scientific development. Nevertheless, the Brazilian Society for the Advancement of Science praised the creation of the new secretaryship. The society lobbied intensely to recreate the ministry in order to have a single entity to coordinate science policy.

Financial problems for science persist. Some ministry institutes now in the new secretary have less than half the money they need to function. Researchers at the National Institute of Amazon Research (INPA) have to buy fuel for their jeeps out of their own pockets. **Ricardo Bonalume Neto**

UK UNIVERSITIES

AUT exam boycott enters week 11

London

THE end of the 10-week-old lecturers' boycott of British university examinations is no closer after weeks of intense negotiations between the Association of University Teachers (AUT), the Committee of Vice-Chancellors and Principals (CVCP) and the government. If the action continues, final year students may not be awarded classified degrees, and universities fear students may take legal action against them. Already mid-term examinations have been cancelled and summer papers are not being prepared.

The CVCP is meeting this week to discuss how to minimize the effects of the strike on students. Vice-chancellors could decide to award unclassified degrees based on tutors' reports or to ask students to sit examinations after the summer, at the time when re-sits are usually taken. Meanwhile, they are hoping that AUT members will disagree with the council, the policy-making body of the union, which last week rejected the CVCP's latest offer of a pay rise. Members will be balloted in April on whether to accept the council's decision.

The offer of an increase of 6.5 per cent for 1989-90 was made after the universities received an undisclosed sum from the government, on condition that the strike is ended. The CVCP had asked for £187 million but received "a lot less than that", according to a spokesman. The union is certain that the universities can finance a larger pay increase, though Sir Mark Richmond, chairman of the CVCP, denies this, saying that the latest offer is final, and that to offer any more would bankrupt some universities.

Christine McGourty

THINK-TANK

Berlin academy fights closure threats

Munich

THE new left-wing government in West Berlin plans to close the 'Academy of Sciences and Technology in Berlin'. The academy was founded in 1987 by the then-conservative government as an interdisciplinary think-tank especially concerned with problems of technology and society. The Greens and Social Democrats now in power denounced the academy as an "old men's debating club" (see *Nature* 329, 659; 1987). The academy's research fields include solar energy, ageing and society, and the impact on German science of the exodus of researchers from Berlin in 1933. It had a 1988 budget of DM8 million, mostly provided by the West Berlin government.

The academy will fight for its existence. Spokesman Eberhard Vogt said that the "last word" had not yet been spoken about the future of the academy.

Steven Dickman

DEFENCE RESEARCH

Independent agency for UK

London

BRITAIN'S main defence research institutes are to be grouped together in an independent defence research agency in order to loosen their ties with the Ministry of Defence, their main customer.

At first the agency will be made up of four institutes: the Admiralty Research Establishment, the Royal Aerospace Establishment, the Royal Armament Research and Development Establishment and the Royal Signals and Radar Establishment.

The Aeroplane and Armament Experimental Establishment is also being considered, but the Chemical Defence Establishment (CDE) at Porton Down will not be part of the agency. A review carried out last year by a study team for the Ministry of Defence (MoD) said that, because of the political and public sensitivity about the work carried out there, and because of the role of the establishment in providing support to government on international arms control, the CDE should be excluded from the agency.

The agency will be expected to increase the work at the institutes carried out for industry, and will have to compete with industry for some contracts for the MoD. There is also likely to be a reduction in staff and in the number of sites. More than

12,000 staff work in the institutes at over 100 sites, most of which are in South-East England where land sales would be remunerative.

There are still many uncertainties surrounding the form of the new agency. A team has been set up to plan in detail the changes before the agency is formed in 1991. It could take the form of a trading fund within or outside the civil service, or it could be a government-owned public limited company. Full privatization has been ruled out.

Manpower is one of the important problems to be tackled. A serious shortfall in recruitment of young graduates, especially in electronics, computing and mathematics, combined with a high rate of resignations among the youngest and brightest of researchers, has led to a shortage of high-calibre staff. The defence research study team said that this could be solved by the introduction of more short-term contracts and by giving the agency more independence to fix pay scales, now set at civil servant rates.

The study team also stressed that in a new agency mechanisms must be put in place to safeguard strategic research, which could be neglected in the commercial climate.

Christine McGourty

US SALARIES

Less pay means empty jobs

Atlanta

THE AIDS epidemic may have helped the budget of the US Centers for Disease Control (CDC) to multiply to more than \$1,000 million a year in the past six years, but the hefty increase has not meant salary rises for its researchers.

Instead, the agency, the front-line of defence against epidemics in the United States which also helps to staunch outbreaks of disease around the world, is suffering from the same problems as the rest of the US government's research enterprise — those of hiring the best and the brightest talent.

Earlier this year, CDC's largest research centre, the 1,000-staff Center for Infectious Diseases, was forced to cast its net outside the United States to fill one of its top posts. Fred Murphy, director of the centre, made the unusual move of hiring one of Britain's top virologists, Brian Mahy, away from his directorship of the Agriculture and Food Research Council's Pirbright Laboratory. Five US scientists offered the job had turned it down because the \$75,000 salary was too low.

Mahy says he was not actively looking for a job when Murphy came knocking on his door, but recent shake-ups in the organization of British veterinary science (see

Nature 338, 191; 1989) made moving to the United States more attractive. He says he considered the CDC post an advancement and it offered him a 25 per cent raise in salary. Several other Pirbright scientists left with Mahy to work at CDC.

CDC's Murphy says he spends much of his time looking for prospective employees, and that it takes 6-12 months to find the right people for executive-level positions within his office and as directors for his centre's 12 divisions. He has had to fill seven "very senior positions" within the past year and half.

The most pressing vacancy at CDC is a replacement for CDC director James O. Mason, who has been chosen by the Bush administration to be the US Assistant Secretary of Health, in charge of the Public Health Service which oversees both CDC and the National Institutes of Health. While Mason or the new Secretary of Health and Human Services, Louis Sullivan, could turn the CDC directorship into a political appointment, George Hardy, acting deputy director of CDC, predicts that the job will be filled by a search committee, a process which could take several months. Mason is sure to take an active interest in selecting the new CDC director.

Carol Ezzell

Fears of drought assuaged

Berkeley & Boston

A MODERATELY wet winter has eased fears of a second year of drought in much of the mid-west and Rocky mountain areas of the United States, and long-range forecasts have even raised hopes of a year of bumper crops. But some regions still face shortages, and despite recent rains, rationing is already being implemented.

Last season's mid-western drought was probably a one-time occurrence, possibly caused by temperature anomalies in the tropical east Pacific. While some areas of the mid-west remain drier than normal, another extensive grain-belt drought is unlikely this year.

Californians now face an unprecedented third consecutive year of drought, as weather patterns in the Pacific diverted the jet stream and its storms away from the state for much of the season. Rainfall for most of the winter was less than half of normal, leaving the state in a dangerously dry condition, according to William Helms of the state drought centre. A wet March has provided some relief in the central part of the state, and the cities of Southern California were spared the full effects of the drought, but much of the San Francisco Bay area is still in crisis and beginning to bargain for water from other parts of the state.

Many Californians assumed the drought could not persist for three years in a row, as it never has in the 120 years during which records have been kept. So the current crisis, with its accompanying water rationing, came as a rude shock. Santa Clara County, home to California's semiconductor industry, is one of the hardest hit areas in the state. Dependent on local water supplies, the county has been little helped by the March rains, which fell mostly to the north and in the mountains to the east.

Reservoirs that feed Santa Clara's underground aquifer were empty in February for the first time in history, sending county officials begging for water loans from other parts of the state, amid fears that land in Santa Clara would begin to sink if more water were drawn from underground.

March rains have eased the plight of California's \$15,000 million agriculture industry, which earlier this season was warned of likely cutbacks in water allotments of up to 60 per cent. The State Department of Water Resources says cutbacks are still likely, but will be smaller.

In the north-east, the unseasonably warm and dry winter has also prompted concern over the prospect of severe water shortages. Water levels in key reservoirs in New York, Massachusetts and several other states are at or near historic low points, after four consecutive years of

abnormally arid weather in the region.

Last week, New York's Drought Management Task Force voted unanimously to recommend that a water emergency be declared for New York City and 11 other counties in the state. The move followed a declaration of water emergency last month in Massachusetts, giving the state government jurisdiction to enforce mandatory restrictions on water usage by municipalities.

Although the situation is most severe in Massachusetts and southern New York, representatives from the United States Geological Survey (USGS) and other state environmental agencies say that all the northeastern states in the United States have been affected by particularly dry conditions over the past year and

many areas in the region are threatened by drought.

In Massachusetts, the declaration of a water emergency came after reports from the Water Resources Authority that the state's largest reservoir, serving more than 40 municipalities, had dropped to its lowest level in 15 years. Similarly, reservoir levels in New York stand at only 55.9 per cent of their capacity, more than 30 per cent below the average for this time of year. Because Massachusetts and New York have had almost no snow this year, state water officials are concerned that the spring thaw will not provide nearly enough water to replenish the system.

The problem in Massachusetts is intensified further by water contamination at some local sources which has forced several municipalities in the state to close their local water supplies.

Marcia Barinaga & Seth Shulman

SOVIET PSYCHIATRY

New independent association

London

NINETEEN Soviet psychiatrists, from Moscow, Leningrad and other major cities, have established the Independent Psychiatric Association (IPA) of the USSR. According to Dr Viktor Lanovoi, president of the new organization, the IPA does not intend to enter into conflict with the existing All-Union Society of Neuropathologists and Psychiatrists. Nevertheless, its founding statement makes it clear that it has come into being in response to the abuses that have compromised Soviet psychiatry in the eyes of the world.

Its founding statement declares that the IPA is open to "all those who consider it necessary . . . to defend the doctor against social and political pressure, and to defend people, healthy or ill, against extreme socio-political and psychiatric arbitrariness".

The IPA founding follows hard on the heels of a two-week visit to the Soviet Union by a team of US psychiatrists to investigate charges of psychiatric abuse. International criticism of Soviet psychiatric practices resulted in a decision in 1983 by the All-Union Society of Neuropathologists and Psychiatrists to resign from the World Psychiatric Association (WPA) to forestall its impending expulsion. The society has now filed an application for readmission, but the IPA has announced its intention to apply to the world body as well, and only one national society in each country can be a member of the WPA.

The US visiting delegation was allowed into the Soviet Union under conditions similar to those governing arms-control inspectors. It visited hospitals and patients of its choice. IPA member Aleksandr Podrabinek, who some 10 years ago

became the first person within the Soviet Union to attempt to monitor psychiatric abuse, worked closely with the delegation. Podrabinek maintains that recent, much publicized "improvements" in the Soviet treatment of penal psychiatric cases were largely cosmetic. Moreover, rather than releasing all political "patients", Podrabinek points out, the authorities have actually hospitalized some new ones.

Nevertheless, Podrabinek welcomed the US visit as an important breakthrough. Now back in the United States, the visiting team will issue an executive summary of its findings in one month. The American Psychiatric Association, one of the trip's organizers, has taken no position on the All-Union's Society's application for readmission to the WPA, and will not comment until the report is produced. But Podrabinek has issued his own account. He stressed the shock felt by many of the Americans at the lack of compassion with which the Soviet psychiatrists treated their political "patients".

Although the Soviet psychiatric establishment clearly needs to win over world opinion, and in particular that of the powerful US vote in the WPA, official opinions, according to Podrabinek, were divided as to whether the proposed US visit would do them more good than harm. Up to the last minute, he said, the Soviet side kept altering the terms of the visit — deciding, for example, that the Soviet psychiatrists and not the patients nor the Americans would select which relatives or friends would accompany the patients during the examinations. In the end, the patients simply brought the relatives of their choice, and the Soviet side made no attempt to challenge these choices.

Vera Rich

NUCLEAR POWER

Holy river no obstacle

New Delhi

IGNORING protests from environmental groups, India last week commissioned the controversial nuclear power reactor at Narora on the banks of the sacred Ganges in the state of Uttar Pradesh, 140 km east of Delhi.

The 230-MW reactor is the first of two units at India's fourth nuclear power station. Commissioning of the second unit at Narora has been put off until 1990 because of a lack of indigenous heavy water.

The twin reactors of the \$500-million Narora Atomic Power Station (NAPS) are based on the Canadian CANDU design that uses natural uranium fuel and heavy water as moderator and coolant. After Canada severed nuclear ties in 1974, India on its own built two CANDU at Kalpakkam near Madras in 1985. NAPS is the second totally indigenous station and, like the Madras reactors, is outside the control of the International Atomic Energy Agency (IAEA). The two US-built boiling-water reactors in Tarapur near Bombay and the two reactors near Kota in Rajasthan, built by Canada, are under IAEA inspection.

The siting of the new power station at Narora has been the subject of a bitter controversy between the Department of Atomic Energy (DAE) and environmental groups such as the Committee for Sane Nuclear Policy (Cosnup). While the Tarapur and Madras stations are sited near the sea, and the Kota reactors use cooling water from a lake, NAPS is the first Indian nuclear plant to be built near a major river. Although only make-up water will be drawn from the Ganges for the closed circuit cooling system, treated effluents will drain into the river, which is currently being cleaned up under a \$250-million 'Ganga action plan'.

Environmental groups have organized a 'Save Narora' campaign that is concerned about possible radioactive pollution of the Ganges, the life-line for 200 million Indians living in its basin. The new reactor at Narora is also the first reactor in India to be located in a seismic zone. Critics say the risk of radioactive pollution of the Ganges is heightened by the fact that the reactors are standing on alluvial soil only 50 km away from the Moradabad Fault which was the centre of an earthquake in 1956.

These fears have been dismissed by DAE, which says the reactors have double containment systems and have been designed for automatic safe shutdown in case of earth tremors. The effluent treatment will be such that there will be 'zero-radiation discharge' into the Ganges. Critics, unconvinced, note that the extra money spent on making the plant earth-

quake-proof could have been saved by siting the plant elsewhere. Earthquake-proof design considerations were mainly responsible for cost and time overruns. NAPS took 12 instead of 7 years to complete and its cost had doubled.

Ironically, commissioning of the new reactor at Narora will not add to India's installed nuclear power capacity but only bring it to the level that existed in 1986. Since then, output from the Tarapur reactors has been deliberately reduced to lessen the radiation dose to personnel there, and one of the two Kota units has been shut down due to a leak from the end plate of the reactor vessel. Attempts to plug the leak permanently have so far failed.

By the year 2000, India plans to produce 10,000 MW or 10 per cent of total electricity demand from nuclear reactors. This would require commissioning of two or three reactors per year, an impossible task. Even if DAE could mass-produce reactors, it has to face a growing anti-nuclear lobby.

Opposition is already mounting against the nuclear station under construction at Kaiga, in the midst of rain forest in western Ghats, and the two 1,000-MW reactors proposed to be built by the Soviet Union at Kodangulam on east coast of south India.

K. S. Jayaraman

Soviet citizens not impressed by IAEA

London

PLANS for a nuclear power and heating station in Gor'kii in the Soviet Union have roused considerable opposition from local inhabitants, who, in the aftermath of the Chernobyl accident, are concerned at the proposed siting of such a station only a few kilometres from the city centre. The Soviet nuclear planners have now agreed to call in experts from the International Atomic Energy Agency (IAEA) in Vienna, to assess the safety aspects. If the IAEA gives its approval, the station could begin supplying the city with heat next summer.

Twelve such stations were originally planned for the European part of the Soviet Union, with Gor'kii as the prototype. But the partly built station at Minsk is already being modified to operate on conventional fossil fuel, under local pressure. And Soviet public opinion, it seems, is not always ready to accept the IAEA experts as disinterested parties. Last autumn, when Dr Murray Rosen of IAEA tried to assure local people that the RBMK (Chernobyl-type) power station at Ignalina posed no threat, he received boos and catcalls.

Vera Rich

CRAFOORD PRIZE

Pioneer in rocketry and radiation discovery

Washington

THIS year's Crafoord Prize has been awarded by the Royal Swedish Academy of Sciences to James Van Allen of the University of Iowa.

The prize, worth about \$250,000, is given annually in an area not covered by the Nobel prizes. This year's recipient is cited for his work in magnetospheric physics, his best-known accomplishment being the discovery in 1958 of the 'Van Allen' belts, regions in the upper atmosphere in which charged particles of solar origin are trapped by the Earth's magnetic field.



Van Allen began his scientific career as an experimental nuclear physicist and became a pioneer of scientific rocketry, designing both novel detectors and the rockets that carried them. The Aerobee rocket was his work, and he was chief scientist for the Explorer I satellite, which found the radiation belts that carry his name. At the University of Iowa in the early 1960s, he and his pupils designed and built an important series of satellites.

These days, Van Allen is known as a critic of manned space exploration, believing it to be a scientifically unproductive extravagance.

David Lindley

FOSSIL LOSS

Tourism falls victim to 'Tyrannosaurus'

Tokyo

JAPAN's only fossil to have acquired the exalted status of a national 'natural treasure' was toppled from its perch last week when word finally reached the press that the carnivorous *Tyrannosaurus* dinosaur discovered in 1976 at Mikasa City, Hokkaido, is actually a lizard, *Mosasaurus*.

Doubts about the fossil's identity have been circulating privately in the Japanese geological community for some time. Now that the truth has come out from the National Science Museum's Ikuo Obata, who made the original misidentification, Mikasa City (population 20,000) is stuck with a massive concrete replica of an erect *Tyrannosaurus*, a thriving industry supplying *Tyrannosaurus* souvenirs — from key rings to sweets and pickles — and a museum containing the fossilized head of what, after all, is a *Mosasaurus*.

City officials are deeply disturbed, saying that the *Tyrannosaurus* business is all they had to stop the outflow of people to big cities. The Cultural Agency, which granted the 'natural treasure' title says it is "studying the situation". Alun Anderson

Reprints still in demand

SIR—Following on the discussion on reprints (*Nature* 336, 708; 1988) I should like to raise two points that very much concern a minority of workers like myself.

First, as a full-time school teacher who has a reasonable output of papers and whose place of employment is not in a university or professional laboratory, I rely on reprints to keep me abreast of my subject. To start with, I rely on a small nucleus of workers who automatically send me reprints of relevant papers. From those, I can then follow up what seem to be useful references by sending for more reprints. I do not have access to abstracting systems nor to libraries that stock journals except on the rare occasions when I make the journey to London.

Second, as an isolated entomologist, I become aware of others with an interest in my field only by receiving reprint requests.

I would be very sad indeed if Ivor Smith's attitude started a widespread movement away from the reprint system.

J.T.C. SELICK

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SIR—Ivor Smith expresses his discontent about the reprint requests he received for his two-page paper, all of which he ignored. He claims that "the European reprint is a disappearing commodity". I believe this attitude is detrimental to at least one part of the scientific community and is inconsistent with efforts to improve cooperation and exchange of information between scientists in the West and East (*Nature* 337, 1; 1989).

I have recently received 153 requests for a one-page paper, 90 of them from countries where neither photocopiers nor scientific journals are abundant. I myself worked for five years in an institute of the Czechoslovak Academy of Sciences. The institute was well equipped and financed and theoretically one could get any paper, but it could take weeks or months, depending on the availability of the journal. It was not possible to order all the papers one wanted that were not available in the library — the capacity of the service would have been far exceeded. Bureaucracy and censorship made things even more complicated: for example, issues of *Nature* were sometimes incomplete. One had to order a copy of a specified article and sign a declaration that the copy would be used only for scientific purposes. Such measures will vanish with the general political shift, but the availability of the scientific literature will not improve significantly in the near future. Reprints remain an important source of information for researchers in the Eastern bloc.

Ivor Smith uses the difference between the cost of postage and that of a photocopy as an argument against reprints. Such reasoning is inappropriate for scientists in the Eastern bloc: as money for postage is limited, some workers pay for this service themselves. This is necessary especially in fast-evolving fields or where more than one field has to be pursued simultaneously.

The decision whether or not to answer a request is a personal one. It depends also on postal funds available. Nevertheless, there is a consensus in the scientific community that reprint requests should be answered. Not to do so is unfair to those who conform — journals that provide authors with free reprints and researchers who ask for them. I suggest, therefore, that authors who cannot or do not intend to send out reprints make an arrangement to spare others the unnecessary costs: as a part of the corresponding address (in order to be included in indexing periodicals such as *Current Contents*), a note "no reprints" should be inserted. This could also serve as a hint for the journals in question to save the costs of producing free reprints.

PETR KARLOVSKY

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SIR—If one wants a copy of a recent paper that has photographs showing ultrastructural detail (common in microscopy and biology journals), then a photocopy is usually not good enough.

PATRICIA A. MOSS

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Sadly confused

SIR—Frank W. Dobbs' criticism, (*Nature* 337, 497; 1989) is sadly correct, as you noted, but he is sadly mistaken on who was confused: it was Kepler who analysed Tycho's measurements and not Copernicus who analysed Kepler's — to wit: Copernicus 1473–1543, Tycho 1546–1601, Kepler 1571–1630.

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

African birds

SIR—Tim Crowe's diatribe (*Nature* 338, 11–12; 1989) on the seventh Pan-African Ornithological Congress (PAOC) left me wondering if we had actually attended the same event. I did not think I was alone in finding it a highly enjoyable, well-organized and scientifically stimulating conference which could also (for the first time, it seems) honestly be described as 'pan-African' in its representation.

It is possible to understand Crowe's frustration over the processing of his and his colleagues' visas. But his obsession with the so-called 'exclusion' of South Africans prevents him from grasping the fact that the future of African ornithology depends on our building a basis for it over the entire continent. In this regard, his dismissal of the papers given by African delegates is particularly unhelpful (not to say offensive). Unfortunately, few African ornithologists will have had access to resources and training of the calibre provided by a FitzPatrick Institute. This makes it all the more important that young scientists have the chance to attend international conferences such as this one, where they can present work, receive constructive criticism and learn from the example of their more experienced colleagues. Indeed, this is one of the best reasons for the congress's new regionalism. The fact that last year's meeting was held in East Africa allowed many scientists to attend who would otherwise never have had the chance, and at future congresses this opportunity will be extended to those in Central and West Africa as well.

Crowe's nostalgia for the days when the PAOC was run by South Africans for South Africans is profoundly unrealistic. I doubt that the absence of many distinguished South African ornithologists can have cheered any delegate to the seventh congress, even among the supposedly "virulent anti-South African elements". But this situation clearly arose as a result of the distortions induced by apartheid, distortions which, while they act to impoverish African science, cannot simply be ignored. The cost of guaranteeing South African participation in all future congresses would be the continued paralysis of pan-African ornithology, and the seventh PAOC decided that this cost is too great. Eventually, the isolation of South African ornithologists must be ended through political change. In preparation, Crowe's colleagues would do well to follow the positive steps outlined at the end of his article — in particular to remedy the disgraceful lack of black South Africans among their number.

LEON BENNUN

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Novel materials from theory

Marvin L. Cohen

The structures of crystals can be predicted using information about their chemical composition as the only input. Such approaches will greatly aid the search for new materials.

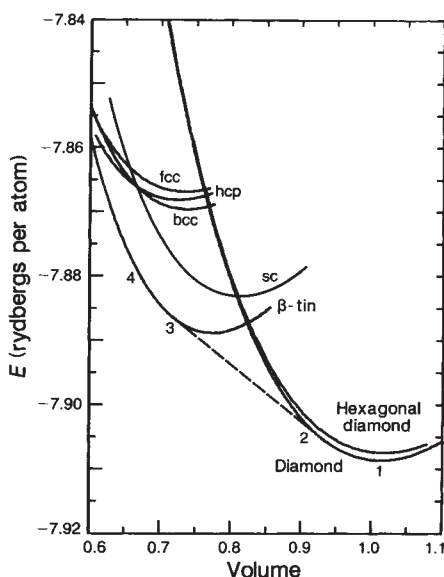
THE "continuing scandal" referred to by John Maddox in his recent article "Crystals from First Principles"¹ is "that it remains in general impossible to predict the structure of even the simplest crystalline solids from a knowledge of their chemical composition". If the words "in general" are intended to mean for all cases of simple crystalline solids, then the statement is certainly true. But for some cases, the implied goal has already been achieved; some of the approaches used strongly resemble what Maddox feels "ought to be possible".

Silicon is probably the best example. At atmospheric pressure and at room temperature, silicon is a tetrahedrally coordinated semiconductor with the diamond structure. In 1980, M.T. Yin and I showed that starting with the atomic number of silicon, 14, a precise estimate of the total energy of solids can be generated composed of specific arrangements of silicon cores of charge +4 embedded in a sea of itinerant valence electrons². A core contains the silicon nucleus and ten tightly held 'core' electrons, which are assumed to be unchanged in going from the atomic to solid state. The four valence electrons from each atom are free to wander throughout the crystal and are expected to arrange themselves in a manner that achieves a minimum energy state. So, for an assumed crystal structure, the total energy of the core and valence electrons could be computed as a function of the core separation, or volume per unit cell.

The next step is that anticipated for future calculations by Maddox. A list of good candidate structures is used to choose various arrangements of the cores, and their energy is compared for many assumed volumes. In the original study² on silicon, seven structural phases are examined (see figure). These are diamond, hexagonal diamond, white tin (β -tin), simple cubic (sc), body-centred cubic (bcc), hexagonal close-packed (hcp) and face-centred cubic (fcc). Several more structures have been considered since 1980. As shown in the figure, the diamond structure was computed to have the lowest energy among these for the volume appropriate for atmospheric pressure. The minimum of the curve of energy versus volume for the diamond structure gives the lattice constant and its curvature yields the compressibility or bulk modulus. The calculated results agreed with experiment to within 0.4 per cent for the lattice

constant and 1 per cent for the compressibility, which is impressive considering that the input is only the atomic number and the list of candidate structures.

This calculation gives a starting point for Maddox's goal that, by the use of a powerful computer, a chemical formula is typed in to "obtain, as output, the atomic coordinates of the atoms in a unit cell". In fact, for silicon even more has been done.



Total energy curves of seven structural phases of silicon as a function of volume normalized to the volume of the diamond phase. The dashed line, which is the common tangent between the diamond and β -tin phase, represents the path for this pressure-induced structural transition. Its slope gives an estimate of the transition pressure. (From ref. 2; see text for further details.)

When the atomic mass was added to the input information, the vibrational spectrum, the electron-lattice interactions, anharmonic lattice couplings, cohesive energy and other properties, together with their pressure dependence, were obtained. But most of these properties were known beforehand. Despite the first-principles nature of the calculation, its success does not completely meet Maddox's challenge — "the goal of calculating from first principles the crystal structure of a compound of which nothing is known except chemical composition".

But examination of the work on silicon again shows that, to a large extent, even this goal has also been achieved. Although the calculation described above demonstrates that diamond is the structure of

lowest energy at atmospheric pressure, its energy increases above that of some other structures as the volume per atom is reduced, which can be achieved in the laboratory by subjecting a sample to pressure. The reduced-volume calculation showed that silicon should be metallic and stable in the white-tin structure at pressures around 100 kilobars and in the hexagonal close-packed structure at 400 kilobars. The path for the diamond to white-tin pressure-induced transformation is shown by the dashed line in the figure. This line is the common tangent between the curves, and the points labelled 1, 2, 3 and 4 represent volumes at which the solid (1) is the diamond structure, (2) is beginning the transition, (3) is ending the transition and (4) is in the white-tin structure. The calculated values of the transition volumes at (2) and (3) are within 2 per cent of their measured values. Using the slope of the common tangent, the transition pressure for the transformation was calculated and it is within several per cent of the measured values. The white-tin transition was known, but the hexagonal close-packed structure was a mere prediction. Experiments were done, and the predicted structure was found at the estimated pressure. The lattice constant and many other properties were also predicted successfully. Another simpler hexagonal structure appeared at lower pressures, and the theory was used to compute its properties.

When the calculations were extended to calculate the lattice vibrational properties and the interactions of the valence electrons with the vibrating core, there was enough information to predict that simple hexagonal silicon should be superconducting at temperatures in the 5–10-kelvin range and that hexagonal close-packed silicon would superconduct around 4–5 kelvin. The predictions were verified^{3,4}, as was the pressure dependence of the transition temperature.

So there is an example of a successful prediction of the existence of high-pressure structural phases, their lattice constants, electronic and lattice properties, and even superconductivity. The calculation proceeds from first principles and requires only the atomic numbers, atomic masses and candidate structures as input. Silicon is the prototype material, but many systems have been studied with similar success. For example, the simple hexagonal structure of germanium was success-

fully predicted to exist in the 800-kilobar range. Other materials that have been investigated include metals, III–V semiconductors and some ionic insulators. Maddox discusses graphite, diamond and ice; the first two have been analysed successfully, but the last has not.

How do these calculations proceed, and what is the state of the art? Maddox focuses on a study⁵ of polymorphs of silica, an interesting calculation which requires input information about SiO_4^{4-} clusters. Maddox describes the achievements of the calculations and mentions some reservations about them. He notes the need to add fictitious positive charges and to set the distances between oxygen atoms and the point charges equal to the observed Si–O distance, for example, observing that it is wrong to say that “everything depends on what the programmers have done”. Most researchers in this area would agree with this observation, but there are approaches which do not call for caveats of this kind.

The present state of this area can be assessed by a more detailed examination of the silicon calculation⁷. The procedure is to compute the total energy by summing the interactions between and among the cores and valence electrons. The core–core repulsion is computed with standard techniques for evaluating the Coulomb electrostatic energy between fixed cores using Madelung sums (the sum of Coulomb repulsions over a whole lattice). The electron–electron contribution to the energy can be estimated directly from the electron density as a function of position in the crystal. The electron–core term is more difficult because the interaction between a valence electron and a core has two main components: the attractive Coulomb interaction and the repulsion arising from the Pauli exclusion principle. Both of these can be incorporated into a pseudopotential which can be constructed for all the atoms in the periodic table and beyond. These potentials are particularly suited to the description of electrons with low angular momenta, but that is not a severe limitation. In principle, solids with many atoms in a unit cell can be studied.

Refined approaches

The progress in this area is well-documented in the literature (see, for example refs 6,7) and many researchers are contributing to the refinement and improvement of these approaches. There is particular interest in the development of schemes to treat excited electron states, to improve the precision of calculating static properties such as cohesive energy and to avoid the need of starting with a list of candidate structures.

There has been significant progress in all three areas. It is now possible to use the first-principles approach to compute optical and photoemission properties of

solids without any experimental input. When electrons are excited by light or heat they interact differently with the other electrons in the solid. Several computations of these changes have been done based on new theoretical approaches⁸ and fairly complex calculational techniques. The refinements in the evaluation of electron–electron interactions beyond schemes which use only the position-dependent density of the electrons are mostly based on extensions of a statistical approach to the encounters between a finite number of electrons. These quantum Monte Carlo simulations⁹ provide the data needed to treat the electron–electron interactions more exactly.

Finally, statistical approaches¹⁰ may allow the determination of crystal structures without tests of candidate structures. By allowing cores to move randomly and in a manner so as to minimize the energy of the entire system, including contributions from the sea of valence electrons, it is expected that the lowest energy structure at a given volume will be the same as nature's choice for that volume. There are problems with this approach, which arise because systems tend to choose local energy minima that are configurations of low energy with core positions close to the starting configuration. These may not be the lowest or global energy minimum, and the solids they predict may be unattainable metastable states of the system. Nevertheless, this problem should be overcome before too long.

In general, the outlook is bright for the development of theoretical approaches with the predictive power envisaged by Maddox. In addition to enabling us to simulate extreme conditions for making materials, with theoretical models we can easily vary the constituent elements in a compound. Unlike experiment, where each change of an element usually requires starting the synthesis all over again, theoretical modelling requires small adjustments and new combinations are tested quickly. The search for new materials using theory can be made more efficient and faster if partially empirical approaches are used. For example, a calculation of the bulk modulus (B) or its inverse, compressibility, for a class of tetrahedral semiconductors requires significant computer time when the first-principles theory is used. But a simpler scaling theory¹¹ gives the results with comparable accuracy using a hand calculator. The formula is

$$B = (1,971 - 220\lambda)d^{-3.5}.$$

By inputting d , the bond length in Ångströms, and the ionicity parameter $\lambda = 0,1,2$ for Group IV, III–V or II–VI semiconductors, respectively, the bulk modulus B in gigapascals is obtained with remarkable accuracy¹¹. The full theory was used to provide the basis and the tests of this simpler approach, but now the

formula for B can be used to give insight into materials development.

Hard as diamond

An example of the use of the formula for B is in the search for materials with hardness comparable with that of diamond. Hardness is generally directly related to the bulk modulus, and diamond has the largest bulk modulus of all materials. It is a tetrahedral material with a very short bond length (d) and zero ionicity ($\lambda = 0$). However, it should be possible to create materials with bulk moduli comparable to or even larger than that of diamond by reducing the bond length. One approach¹¹ is to attempt a synthesis of tetrahedrally coordinated insulators using carbon and nitrogen because the C–N bond length could be shorter than that of diamond. Even though a material of this kind will be partially ionic, the shorter bond length can over-compensate and increase B . Boron nitride is another superhard material of interest. The results for B using the above formula and a complete first-principles theory predicting B and the equation of state for boron nitride were tested¹² recently by direct experimental measurement. The predictions were found to be in excellent agreement with experiment.

Although the development of less-complex empirical approaches is likely to have a great influence on the development of useful materials, the emphasis at present is on the application and refinement of the first-principles theory. Research on the possibility of compressing hydrogen into a metallic state, the possibility of high-temperature superconductivity for metallic hydrogen, the existence of superhard systems and the general search for useful new materials can be aided by first-principles theory. Some materials have already been identified in this way and it is likely that more will be found as a result of theoretical predictions. □

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Soap bubbles make serious physics

A new study of the evolution of rafts of soap bubbles confirms expectations and earlier measurements, but has the distinction of having been carried out with a photocopying machine.

USING a Xerox or some other brand of photocopying machine, with virtually no extra equipment, to make interesting physical observations is an achievement in itself. But Joel Stavans from the University of Pittsburgh and James A. Glazier of the University of Chicago seem also to have won some interesting physics from their primitive equipment (*Phys. Rev. Lett.* **62**, 1318; 1989). The issue is the evolution in time, by coalescence and other means, of a raft of soap bubbles which are small to begin with, but which get larger with the passage of time.

As with so many other topics, that of the evolution of a bubble raft goes back at least to J. von Neumann, among other things the author of a law describing the evolution of a single bubble embedded in a raft of them. It is important that, in circumstances such as these, bubbles are not circular or spherical but, rather, sit like prisms on polygonal bases. The neat way of fixing ideas is to imagine the experiment which Stavans and Glazier describe: put a raft of coloured soap bubbles in a very shallow plastic tray and seal a lid on top in such a way that the soap films stretch between the flat and horizontal upper and lower surfaces, giving each bubble (to a first approximation) the same height. Liquid collects at the lower boundaries between contiguous bubbles, so that the evolution of the bubbles in a raft may be followed simply by pressing the button on the photocopier at convenient intervals.

A bubble will grow or shrink by the diffusion of supporting gas from all nearest neighbours, which leads directly to von Neumann's law of bubble-growth that the rate of change of the volume of a bubble (or of its area as seen in a photocopied image) depends only on the number of vertical faces it presents to its neighbours in the raft, or on the number of sides of the polygon which forms the base. The starting-point for the argument is that a strictly regular array of bubbles each of which is hexagonal should be indefinitely stable, which leads directly to the rule $da/dt = \kappa(n - 6)$, where a is the projected area of the bubble, n the number of sides and κ a kind of diffusion constant.

The simplicity of this result is geometrical, deriving from the way in which both that rate of inward diffusion and the volume of the prism is determined by the total area of the vertical faces of the prismatic bubbles. A further assumption

is that the internal angles of each polygonal bubble-base are exactly 120° , which seems reasonable enough, given that each vertex of the polygon is a point at which three prismatic bubbles join; to a first approximation, they may be assumed to join symmetrically. But naturally this can be exactly true only when all bubble bases are regular hexagons. Otherwise, the sides of the polygonal bases will not be straight lines, but curves.

Stavans and Glazier are not primarily concerned with single bubbles, but with rafts of them, for which purpose they work with a distribution function called $q(n)$ which gives the frequency in any sample of the evolving bubble raft of bubbles with n sides, where n is necessarily an integer running from 3 to infinity.

Simple observation is said to show that the first stages of the evolution of the raft are rapid, with larger bubbles growing at the expense of smaller neighbours and the sidedness of particular bubbles changing upwards and downwards both by the disappearance of smaller neighbours and by a process in which vertical membranes shift so as to trade sides between one bubble and a neighbour. (Those planning to use their office photocopier for further investigations along these lines should beware of the edge effects, which complicate both the statistics and the underlying phenomenon.)

The authors explain how, when they made soap bubbles with helium gas, the general appearance of the raft thus formed was that of several patches of regular hexagonal bubbles interspersed with regions in which five and seven-sided polygons were mixed together. But the striking result, confirming earlier conjectures, is that there comes a point in the evolution of a bubble-raft in which the distribution function $q(n)$ becomes essentially invariant in the course of time. Moreover, the mean of the distribution (that is the average number of sides per bubble) is almost but not quite six, whatever the length of time for which the raft has been evolving (in one run, indeed, measurements extended over a day and a half). Only when the total number of bubbles becomes very small does the distribution go awry.

The same point emerges from measurements of the second moment of the distribution (essentially the weighted average of the squared deviation of the number of sides per cell from the average

value). Again, in at least two different systems, the value of the second moment fluctuated widely to begin with, but then quickly settled down to what seems to have been a constant value (estimated at 1.4 ± 0.4). The interest seems to be that, as in fractal and chaotic systems, there seems to be an underlying scaling law which describes how, in an ageing raft of bubbles, the bubbles all get bigger, but the statistical properties of the quantity appropriately called sidedness remain essentially constant.

Departures from the rule that the internal angles of the basal polygons of each bubble should be 120° excite particular interest. What the measurements show is that bubbles with fewer than six sides have internal angles smaller or equal to 120° , but that bubbles with more sides have larger angles (although 130° seems to be the limit). Stavans and Glazier argue that the presence of angles differing from the expected value implies the accumulation of strain energy in the system, which allows them to generalize von Neumann's law (but only for those systems in which all the bubbles have the same number of sides) to take account of these departures from the expected. One practical conclusion seems to be that it is possible to understand why one bubble may grow at the expense of a neighbour with the same number of sides.

There remains one puzzle. Perhaps the simplest measure of the state of an ageing bubble raft is the average bubble area, which should, by simple integration of von Neumann's rule, be proportional to the time elapsed. But neither Stavans and Glazier nor their predecessors in the field have been able to measure anything like that — they quote a figure of 0.59 ± 0.11 , as the exponent in a simple power-law dependence on the time.

Why should such a simple prediction apparently be so wide of the mark? It is natural that people should have gone haring after fanciful explanations to do with the scaling laws as bubble-rafts age. Prosaically, the authors suggest a more mundane explanation — that as a raft ages, more soap solution accumulates beneath the raft while there are thicker layers of it between membranes, the effect of which is to reduce the diffusion constant between neighbouring bubbles. It all goes to show that there are merits in even the least reliable photocopies.

John Maddox

Dissecting a molecular motor

Peter Hollenbeck

THE organelles undergoing directed transport within eukaryotic cells are a strikingly diverse group. They vary widely in function and composition — consider membrane vesicles, pigment granules, mitochondria, and nuclei, for example — and range over at least two orders of magnitude both in their size and in their velocity of movement. Given this variation in the cargo, it is no surprise that cells use several different mechanochemical systems to generate force for movement. Two of these are well-characterized: myosin ATPase, which drives movement along actin filaments; and dynein ATPase, which generates unidirectional movement towards the 'plus' ends of microtubules. A third, kinesin, has not yet been so well characterized. On page 355 of this issue¹, Scholey *et al.* describe the coordinated use of protein biochemistry, molecular genetics, antibody probes and electron microscopy to analyse the properties of kinesin, which should allow a comparison of these mechanochemical enzymes.

Although they interact with different filament systems, and have different kinetic properties, myosin and dynein have some interesting similarities². In addition to a common structural motif (see figure), they share a crossbridging mode of force generation; they bind to the structure to be moved, thus crosslinking it to a filament; and hydrolyse ATP, transducing the energy thus liberated into movement along the filament. Several groups have dissected the kinesin molecule in attempts to fit its mode of action into this scheme, and Scholey *et al.*¹ are now able to assign the various activities of the kinesin molecule to different structural domains. They find that kinesin has an architecture very similar to myosin and dynein.

Kinesin, whose discovery and characterization I have discussed previously in News and Views^{3,4}, is a ubiquitous protein motor which generates movement along microtubules in the opposite direction to dynein, and is believed to be involved in anterograde (or centrifugal) organelle transport. It is a heterotetramer, each molecule consisting of two ATP-binding heavy chains of relative molecular mass 110,000–140,000 (110–140K) and two 60–65K light chains^{5,7}.

It appears in the electron microscope to have two globular heads at one end of a narrow stalk, similar to dynein and myosin, as well as a smaller pronged or fan-shaped region at the other end of the stalk⁸.

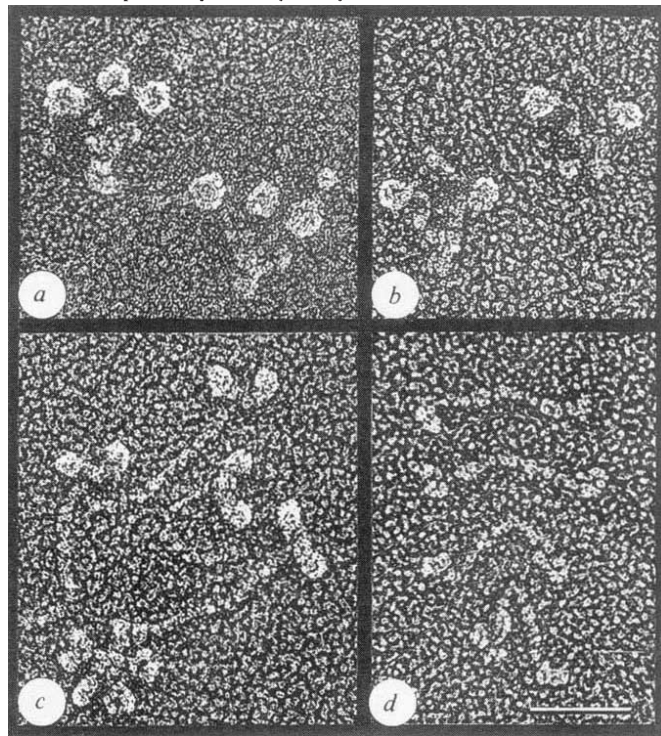
Degradation of the heavy chain with proteolytic enzymes yields a resistant 45K

The studies by Scholey and collaborators reported in this issue¹, as well as recent work by Hirokawa *et al.* reported in the current issue of *Cell*¹¹, combine several experimental approaches to map the location of specific functional domains on the kinesin molecule. Scholey *et al.* use freeze-etch electron microscopy of kinesin to show that an antibody which binds the 45K heavy-chain fragment and inhibits kinesin motility 'decorates' only the paired globular heads of the kinesin molecule. In addition, they use a series of

truncated kinesin heavy chains (produced by transcribing and translating truncated complementary DNA encoding the heavy chain) to demonstrate that the 45K fragment represents the amino-terminal one-third of the molecule, and that the inhibitory antibody binds near the carboxy-terminal end of the fragment, where the globular and α -helical regions of the heavy chain meet. In a related study, Yang *et al.*¹² confirm that the amino-terminal portion of the heavy chain is necessary for microtubule binding.

Using quantitative morphometry of rotary-shadowed kinesin, Hirokawa *et al.*¹¹ show that monoclonal antibodies specific for the kinesin heavy chain decorate only the globular head regions, whereas those specific for the light chain decorate the fan-shaped region of the molecule at the opposite end of the stalk. These authors also report ultrastructural evidence that the globular heads interact with microtubules, whereas the feathered region, and perhaps a portion of the stalk, interacts with the moving structure.

A coherent picture of kinesin is emerging from these studies: the amino-terminal region of the heavy chains, residing in the globular heads, contains the microtubule-binding and ATP-hydrolysing capacities of the molecule; a flexible stalk connects this business end to an organelle-binding domain; and a domain important for the coupling of ATP



Electron microscope images of 'motor' molecules prepared by adsorption to mica, freeze-drying, and replication with platinum according to Heuser¹⁴. *a*, Two 3-headed ciliary dynein molecules prepared from *Tetrahymena* by U. Goodenough; *b*, two 2-headed cytoplasmic dynein molecules prepared from chick cells by E. Steuer; *c*, four myosin molecules from *Acanthamoeba*, one of which is attached to an anti-tail monoclonal IgM molecule (the asterisk-shaped entity in the lower left) (D. Kiehart); *d*, four kinesin molecules prepared from chick brain tissue by T. Schroer. Bar, 40 nm. (Courtesy of John Heuser.)

fragment which provides an important key to kinesin's fine structure. Kuznetsov *et al.*⁹ analysed this fragment in detail and determined that kinesin's ATP hydrolysing and microtubule-binding activities reside there; in fact, the fragment shows elevated levels of both activities relative to the intact molecule. It is significant, however, that the fragment is not capable of generating movement in an *in vitro* assay. Ingold *et al.*¹⁰ demonstrated that the fragment contains the binding sites for several monoclonal antibodies which inhibit kinesin-driven movement *in vitro* without inhibiting kinesin's microtubule-activated ATPase activity. This suggests that the fragment contains at least two functional domains — the ATPase site and an additional region distinct from the ATPase site but nonetheless essential for force generation.

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hydrolysis to force generation lies near the junction of the globular head and the stalk. The complementary DNA sequence of the heavy chain, determined by Yang *et al.*¹², provides additional support for this picture; it predicts a large amino-terminal globular region containing an ATP-binding sequence, connected to a small carboxy-terminal globular region by a long stretch capable of forming an α -helical coiled-coil¹². This sequence also shows a proline-glycine break at approximately the region where a kink appears in electron micrographs of the stalk^{8,11}.

So far, so good — but studies in which

organelle movements have been reconstituted *in vitro* from cell fractions suggest that a further functional domain of kinesin awaits identification. Schroer *et al.*¹³ have shown pure kinesin alone is not enough to produce movement of salt-stripped membrane vesicles; additional factors are required. The sites of interaction between these potential regulatory factors and the kinesin molecule remain to be found. □

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SUPERNOVA 1987A

The remnant's rapid heartbeat

M. A. Alpar, I. Fushiki, F. K. Lamb, G. S. Miller, M.-G. Park and D. Pines

THE supernova explosion of February 1987 in the Large Magellanic Cloud has so far provided a wealth of data which, at least in broad outline, has confirmed our expectations of such an event and its aftermath. As the diffuse remnant has expanded and faded, astrophysicists have watched with mounting anticipation for any indication of the pulsar expected to have been formed in the explosion. The report by Kristian *et al.* in last week's issue¹ of optical pulses with a period of 0.5 ms is the first indication of such a pulsar. This report has aroused enormous interest among astrophysicists, as illustrated by the papers by Woosley and Chevalier² and Wang *et al.*³ on pages 321 and 319 of this issue. It challenges almost all the main features of current theories of neutron-star formation, structure and evolution, and, most fundamentally, current models of nuclear interactions and the equation of state of matter at the highest densities. It seems to us that there are three possible interpretations of these results.

The most interesting possibility is that the pulses are produced by a neutron star rotating with a period of 0.5 ms, three times faster than any previously discovered. If so, this would strongly challenge the picture that has emerged from 20 years of research on neutron-star structure and the equation of state of dense matter. The near consensus has been that pulsars such as the Crab and Vela pulsars are spinning neutron stars of about 1.4 solar masses ($M_{\odot}$) and with radii of 10–15 km. This reflects an equation of state describing neutron matter that is moderately stiff.

Models of pulsar glitches and post-glitch relaxation, the interpretation of the 35-day cycle of Her X-1 as neutron-star precession and deductions of the nuclear interactions from heavy-ion collisions⁴, all suggest that the equation of state of dense matter is relatively stiff. The only uniformly rotating models that predict neutron stars that are sufficiently compact

not to fall apart with a 0.5-ms rotation period are based on soft equations of state: the simultaneous demands that the equation of state be stiff enough for slowly rotating neutron stars with gravitational masses as high as $1.4 M_{\odot}$ to exist and soft enough to permit the existence of a neutron star with a 0.5 ms rotational period are very constraining. The evidence for a stiff equation of state might be reconciled with a rotational interpretation of the reported pulsar if it had a very high central density, and if matter undergoes a phase transition at such high densities. This would then leave the question: why should SN1987A have left a particularly dense remnant?

The 0.5-ms rotation period would also constrain the dipole magnetic field strength B at the neutron-star surface. Electromagnetic dipole radiation has a power output proportional to $B^2\omega^4$, where ω is the rotation rate. For this power to be less than the 10^{35} ergs s⁻¹ current total luminosity of the supernova remnant, the surface dipole field must be less than about 10^9 gauss (G). The magnetic fields of all of the relatively young (but not newborn) pulsars, of which the 1,000-year-old Crab pulsar is the paradigm, are estimated to be 10^{11} – 10^{13} G. The pulsar implied by the report of Kristian *et al.*¹ would resemble these familiar objects only if the magnetic field can increase by a factor of about 10^3 within a thousand years — a possibility that was suggested some years ago, but which is highly speculative.

The alternative assignment of such a weakly magnetic pulsar to the class of other millisecond pulsars which have typical field strengths 10^8 – 10^9 G challenges the widely accepted view that such pulsars are formed in low-mass binary systems (see the discussion by F. Graham-Smith⁵ in News and Views last year). It is thought that such pulsars previously had periods of the order of seconds, and that their strong primordial fields have decayed. The combination of a weak magnetic field and

a high rate of accretion from the partner object in such binaries allows the neutron star to be spun up to a millisecond period. The periods, magnetic fields and ages of millisecond pulsars and rotation-powered pulsars in binary systems are consistent with this model. The location of some millisecond and binary pulsars in globular clusters, and the view that the fast quasi-periods observed in the low-mass X-ray binaries result from 10^9 -G magnetic fields in neutron stars rotating with millisecond periods, also fit this model.

It is conceivable that the weak magnetic fields in these seemingly old systems are primordial and do not result from the decay of stronger initial fields. Subsequent rapid accretion would then leave the star rotating with a millisecond period. But if the newly identified object in SN1987A is a newborn pulsar with both a weak magnetic field and a short spin period, this would undermine the model of spin up by accretion.

Woosley and Chevalier in this issue² propose that the pulsar was spun up to its millisecond period in the hours immediately following the supernova explosion. For this to occur, the neutron star's magnetic field must act to transfer angular momentum from the supernova envelope to the neutron star in a way similar to that supposed in the model for accretion from a binary partner. This explains, the authors suggest, why the new pulsar has the same field-period correlation as the other millisecond pulsars. But the high accretion rate necessary in this model means that the mechanism for generating the observed pulses must be different from that in canonical pulsars. Also, one might expect the accreting material to smear the pulses by scattering.

If the 0.5-ms period is established to be a rotational period and this pulsar turns out to be in the same class as the previously known millisecond pulsars, a fundamental revision of our ideas of pulsar classes, statistics and evolution will be required. Given a neutron star with such an unusual structure and spin period, the natural question for astronomers will be whether the progenitor star and the supernova event itself were peculiar. Do such blue giants have unusually weak magnetic fields, rapid rotation rates or peculiar internal structures? Even if systematic differences are identified, supernova theorists will face the challenge of linking them to distinct features of the stellar core that collapses to become the neutron star.

An alternative possibility proposed by Wang *et al.* in this issue³ is that the neutron star is ringing, or vibrating, with a period of 0.5 ms. A particular attraction of this idea is that it does not require a new and different kind of object or a radical revision of our current understanding of neutron stars and pulsars. Indeed, a 0.5-ms radial vibration would be typical of neutron stars

with reasonable masses and equations of state. Historically, neutron-star vibrations were rejected as an explanation of pulsars because vibration periods are much shorter than the vast majority of observed pulsar periods, and because the observed pulses must persist for thousands to millions of years. Such persistence is natural for neutron-star rotation, but difficult for vibrational oscillations, which are expected to be damped within a few hundred years. However, this need not pose a problem for a newborn pulsar, because radial modes excited at the time of formation may not yet have been damped.

Wang *et al.* propose that some of the vibrational energy is converted into the observed light pulses by the formation of a shock front as the vibrations propagate into a thin surface region of the star where the density and sound speed decrease very sharply. The model requires the shock to accelerate iron ions to energies of 100 GeV; the ions subsequently emit cyclotron radiation without losing much of their energy to electrons or lighter nuclei. A surface magnetic field of 10^{12} G is needed for the iron ions to emit radiation in the optical band. Light nuclei and electrons would be relativistic at 100 GeV, and hence would require lower magnetic fields to emit predominantly in the optical; their synchrotron lifetimes would then be longer than the observed pulse period, washing out the pulsations. Wang *et al.* postulate that the field is 10^{12} G, in which case the rotation period must be longer than 0.1 s or the dipole radiation power would be more than the current supernova luminosity. Stable polarized pulses indicating rotation with a period in this range would provide strong support for the radial-oscillation hypothesis.

Confirmation of the vibrational mechanism, which would not challenge the existing theory, would focus new attention on aspects of neutron stars such as the structure of their surface layers, the propagation of radial oscillations and shocks, and the coupling of such motions to rotation, non-radial modes and possibly to gravitational radiation. There would also be renewed interest in the generation of optical pulses, either through the mechanism proposed by Wang *et al.* or by other means. Nevertheless, it is hard to see why the harmonic content of the observed pulses should closely match that of the Crab pulsar, if the pulsation mechanism is indeed so different. In particular, if the pulse has two peaks, a main pulse and an interpulse, as is suggested by the relative strengths of the fundamental and first harmonics, then this may be difficult to model with vibrationally driven pulsations.

Whatever the explanation for the pulses, there seems also to be an 8-hour sinusoidal modulation of the pulse period,

which poses additional problems. Precession of the neutron star cannot account for the large phase offset observed. Proposals that the modulation is the result of timing noise must confront the comparatively noiseless sine curve of the observed data: it is possible, but unlikely, that this has been produced by a random process. The idea that the modulation arises from a Doppler shift as the pulsar orbits a small, unseen companion, raises the problem of explaining how a companion comes to be so close to the neutron star. It is very unlikely that material left over from the progenitor star would be able to cool and condense into a gravitationally bound companion. Had the companion existed independently before the explosion, it should have had to exist within the progenitor star. But perhaps a companion was produced as the result of spin-up of the core during the supernova implosion.

The third possibility, and the least appealing one, is that the observations are

METEORITES

Unique find from Antarctica

Robert T. Dodd

METEORITES, it is generally agreed, are mostly pieces of pre-planetary material that both come from and were formed in asteroidal parent bodies. This interpretation makes meteorites uniquely important as witnesses to events in the primitive solar nebula, but it also raises a serious question about how typical they are of nebular material. Well-classified meteorites come from no more than 20 of the thousands of asteroids that have been described. Worse, spectral reflectance comparisons suggest that the most abundant meteorites — ordinary, or common, chondrites — come from very rare asteroids: the dark objects that are most numerous in the asteroid belt have, in contrast, yielded few meteorites. So it seems that our sample is incomplete and strongly biased towards those asteroids that are now well-placed to deliver meteorites. If so, meteorites recovered from the Antarctic ice cap, some of which fell a million or more years ago, may both introduce us to new kinds of meteorites and provide fresh insights on old problems. Allan Hills 85085, a thumb-sized Antarctic meteorite described recently in three consecutive papers in *Earth and Planetary Science Letters*¹⁻³, does both.

Despite suggestions to the contrary⁴, ALH85085 seems to be a chondrite: it contains both the spherical-to-rounded objects — chondrules — from

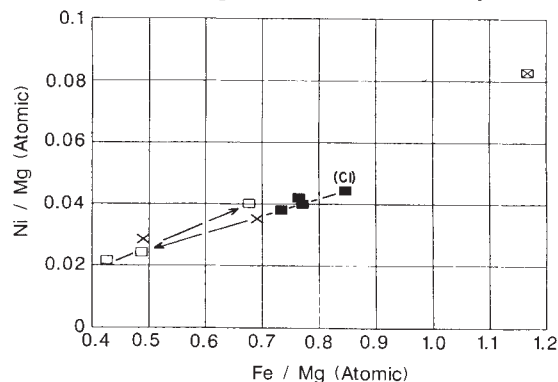
not what they seem, and that no pulsar has actually been detected, despite the strong signal observed and careful analysis. This would save us the trouble of revising our cherished beliefs, but would leave us wondering uncomfortably what was observed, if not a pulsar? Still, until the report of Kristian *et al.* is confirmed by further observations, this is a possibility that must be considered. Should the report not be confirmed, the questions which it has raised may well remain unresolved. This would surely be a disappointment, in view of the exciting challenges the initial report has posed. □

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which such meteorites take their name and inclusions of highly refractory calcium–aluminium silicates (CAI) like those found in many carbonaceous chondrites. It is, however, sufficiently different from other chondrites to make its classification difficult. Its chondrules are few, small, and accompanied by much fragmental material. According to Scott¹ and Weisberg *et al.*², its silicate particles are very closely sized, and the associated oblong metal particles appear to have a preferred orientation².

ALH85085 lacks the extremely fine-grained, dark matrix material that surrounds chondrules in other primitive chondrites, but it contains lumps of similar material which at least resemble^{1,2} and, according to Grossman *et al.*³, may in fact



Metal–silicate fractionation trends in chondrites. Mean ratios have been normalized to the Si/Mg ratio in CI chondrites to remove the effect of refractory element fractionation. Data from refs 3 (ALH85085) and 7 (chondrite groups). ■, C groups; ×, E groups; □, O groups; □X, ALH85085.

be fragments of chondrule-poor carbonaceous chondrites. The meteorite also differs from other chondrites in chemical composition^{1,2}; it is enriched in iron, nickel and other siderophile elements and is deficient in sulphur, alkalis and other elements that vaporize at low temperatures (volatiles).

Those who have studied ALH85085 agree that it is a unique meteorite, whose composition and mineralogy suggest that it is most closely related to two unusual carbonaceous chondrites, Renazzo and Al Rais. Whether it should be grouped with those meteorites³ or left unclassified^{1,2} is less certain. An oxygen isotope analysis or a cosmic-ray exposure age, neither of which is now available, may answer that question.

Of the many intriguing features of ALH85085, its enrichment in siderophile elements and depletion in volatile elements are most significant, for they seem to answer a long-standing question. Almost two decades ago, Larimer and Anders proposed⁴ that other kinds of chondrites evolved from material similar to the chondrule-free CI carbonaceous chondrites (*sic*) by extraction of metallic nickel-iron and refractory calc-aluminous silicates.

Their interpretation, perhaps modified to allow for two episodes of metal fractionation⁵ (see figure), is plausible and widely accepted, but it leaves a great deal of siderophile material unaccounted for. Hence the discovery of a chondrite that contains excess metal of about the right composition² is important, both for the chemical evolution of chondrites and for the origin of other objects — the Earth and Moon included — that also appear to have formed from atypical chondritic material³.

To date, most meteorites from Antarctica have turned out to be samples of known types or modest variations on familiar themes. If the Earth receives a biased sample of the meteorite sources, it seems that it has done so for at least a million years. Yet the ice cap has already yielded many unusual and important objects, which include the first lunar meteorites and additions to a small collection of meteorites from Mars. ALH85085 shows that even its smallest gifts may open new windows on the origin of the Solar System. □

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Is grandmother an oscillation?

Michael P. Stryker

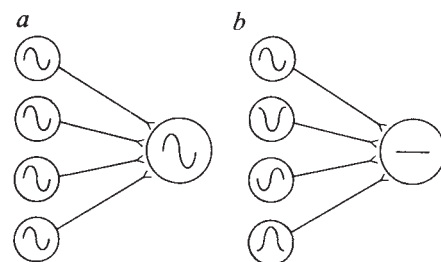
THE realization that visual information is processed through successive stages in many separate areas of the cortex has led to a dilemma. How are the distributed representations of the visual features that have to do with a single object in the world put together so that they can create a perception or influence action? That is, when a green furry tennis ball and an ebony billiard ball are seen in the same part of the visual field, how can the colour, motion and texture properties of each ball be associated, so that the black colour and smooth texture of the billiard ball is not ascribed to the tennis ball? Three new papers¹⁻³ from two laboratories, one on page 334 of this issue², provide a clue.

Several proposals have been put forward to solve this problem. A now-classic notion is that significant combinations of features are hierarchically extracted and combined in specific cortical areas specialized for the recognition of certain classes of objects^{4,5} or, at the extreme, in the receptive fields of single neurons⁶. In some higher cortical areas, there could be, for example, 'grandmother' neurons responding selectively to the precise combinations of visual features that are associated with one's grandmother. Either such cells would not be able to signal the location of stimuli very accurately or there would have to be a separate grandmother cell for each region of the visual field. It is also not clear how the many different features that might be associated with one's grandmother could be combined in any very selective way without a 'combinatorial explosion' in the numbers of cells required⁷. This would lead to the sort of problem Little Red Riding Hood had when her grandmother cells failed to discriminate the wolf's grey fur, sharp white teeth and heavy breathing from her grandmother's normal benign appearance.

These shortcomings have led to an alternative notion — that the representations in the brain of various visual properties of objects in the world are combined only transiently, rather than in fixed receptive fields, in some way that makes the conjoint output of different property-specific detectors available to the mechanisms for perception or action. Some years ago, Crick⁸ suggested a mechanism by which the neurons of thalamic reticular nucleus below the cortex could unify the perceptual qualities represented in different cortical areas. He proposed that a neural 'searchlight' would simultaneously illuminate all the neurons that are activated by the same object in the world.

The recent work from Gray, Singer and colleagues, reported in this issue² and else-

where¹, and from Eckhorn and colleagues³, raises a related possibility: that neurons in the visual cortex activated by the same object in the world tend to discharge rhythmically and in unison. Such a one-note neural harmony could, in principle at least, provide the neurons at higher cortical levels with stronger inputs so that they associate the activities of lower-order neurons with one another (see figure). If the discharges of the texture-, colour-, depth- and movement-sensitive neurons



Four lower-order neurons providing input to one higher-order neuron. *a*, Neuronal activity in the lower-order neurons is shown oscillating in phase. The resulting postsynaptic potentials sum in the target cell, producing a large oscillation in its membrane potential. At the peak of this oscillation, the membrane potential of the higher-order neuron would exceed the discharge threshold, and the cell would fire rhythmic, high-frequency bursts of spikes. *b*, The activities in the lower-order neurons do not oscillate in phase, so the higher-order neuron receives a more nearly constant input. The resulting steady membrane potential in the higher-order neuron either would be below the threshold for spike discharge or would, in any case, not allow it to discharge at the high frequencies characteristic of neuronal responses to sensory stimuli.

concerned with the tennis ball were to oscillate in phase with one another and out of phase with the billiard ball responses, this might enable perceptual mechanisms to assign the furry texture, green colour and blinding speed to the one object and the smooth texture, ebony colour and moderate speed to the other. I have made a simple estimate that, with reasonable assumptions about the duration of postsynaptic potentials, such a mechanism would enable higher-order cells to distinguish inputs from one set of neurons from those of 10 or more sets of neurons, if each set responds to different objects in the world at different phases or frequencies. If, instead of summing, synaptic inputs can interact multiplicatively⁹, cortical cells could detect phase-locked activity with even more sensitivity.

This new evidence provides only the first hints that the visual cortex uses such mechanisms. Gray and Singer¹ find that visual stimulation can cause many neurons in visual cortex to discharge their action

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potentials rhythmically at about 40–50 Hz in very lightly anaesthetized (and, as reported in an abstract elsewhere, awake) animals. This rhythmicity is accompanied by an oscillation in the extracellular field potential and seems to originate in the cortex, as it was not evident in recordings from the main input to the visual cortex from the thalamus. Eckhorn and colleagues³ find that oscillatory field potentials evoked by some visual stimuli are in phase even between the two primary visual cortical areas (17 and 18) in the cat and that the field potentials in one area are in phase with the action potential discharges in the other. In their paper in this issue², Gray *et al.* report that oscillations in the discharge are commonly in phase for neurons with overlapping receptive fields, irrespective of their selectivity for a particular stimulus orientation¹⁰. But when the authors record at sites more than 2 mm apart, where receptive fields no longer overlap, they find that oscillations are rarely in phase except for neurons with the same orientation specificity.

The most surprising observation comes from two cases in which pairs of recording sites were located half a visual cortex (7 mm) apart. Receptive fields of the neurons at the two sites had a common orientation specificity and were aligned so that they could be stimulated by a single long bar of light (see Fig. 3b of ref. 2 on page 336). Neuronal discharges at the two sites were well correlated only when the cells were stimulated with a single long bar. When they were simultaneously activated by two shorter bars that did not bridge the gap between the two receptive fields, the correlation disappeared. In that sense, the correlated discharge can be considered as depending on the global property of the stimulus — whether it is a single or two different objects.

Numerous questions are raised by these tantalizing observations. Where does the rhythm come from? Many workers have dismissed findings of rhythmic discharge in the central nervous system as artefactual, as rhythmicity can be induced by certain anaesthetics or by damage. Further work demonstrating that such phenomena are definitely present in awake animals will be needed to satisfy these critics. Even if not an artefact, the oscillations may be epiphenomena:

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2. Gray, C.M., König, P., Engel, A.K. & Singer, W. *Nature* **338**, 334–337 (1989).
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observations of rhythmicity and its correlation with particular stimuli do not allow the conclusion that the nervous system makes use of such information. This point will be difficult to address except by demonstrating that many global aspects of visual stimuli are accompanied by correlated rhythmic discharges. In particular, it would be intriguing to know whether a single bar or edge produces such correlated activity when and only when it appears as a single object; a physical discontinuity that does not degrade the perception of the bar as a single object, as when the bar is partially occluded¹¹, should not destroy the correlated oscillations. It will be even more intriguing to see whether neurons in the different cortical areas specialized for colour, depth, motion and so on exhibit correlated, rhythmic discharges when

PRIONS

Sheep disease in human clothing

Charles Weissmann

THE identification of polymorphic markers linked to a genetic disease is an important goal both for geneticists and for molecular biologists. The former make use of the association to determine individuals at risk for the disease, the latter to hunt for the gene causing it. Because it is based on trial and error, the search is often protracted. But this is not the case in the familial (ataxic) form of Gerstmann-Sträussler syndrome, a transmissible neurodegenerative disease so rare that it is not mentioned in the human geneticist's bible, *Mendelian Inheritance in Man*¹. Guided by their work on scrapie, a related brain disease of sheep, Prusiner and colleagues, as they report on page 342 of this issue², have in one fell swoop identified a polymorphic marker tightly linked to this syndrome. At the same time, these authors provide persuasive evidence that a surface membrane protein in neurons called PrP has a central role in the pathogenesis of the human disease.

Gerstmann-Sträussler syndrome and Creutzfeldt-Jacob syndrome in humans, as well as scrapie and bovine spongiform encephalopathy in animals, belong to a class of slow, transmissible, lethal brain diseases caused by an unusual kind of pathogen which Prusiner³ called a prion. The main pathological features of prion-associated diseases are vacuolation of neurons, proliferation of glial cells and occurrence of amyloid plaques containing a form of PrP. The diseases have extended incubation times that can be greater than 30 years.

Scrapie has been used as a model for prion diseases because it has been transmitted to hamster and mouse, making it suitable for experimentation. Because scrapie infectivity is very resistant to heat

they respond to the same stimulus in the real world.

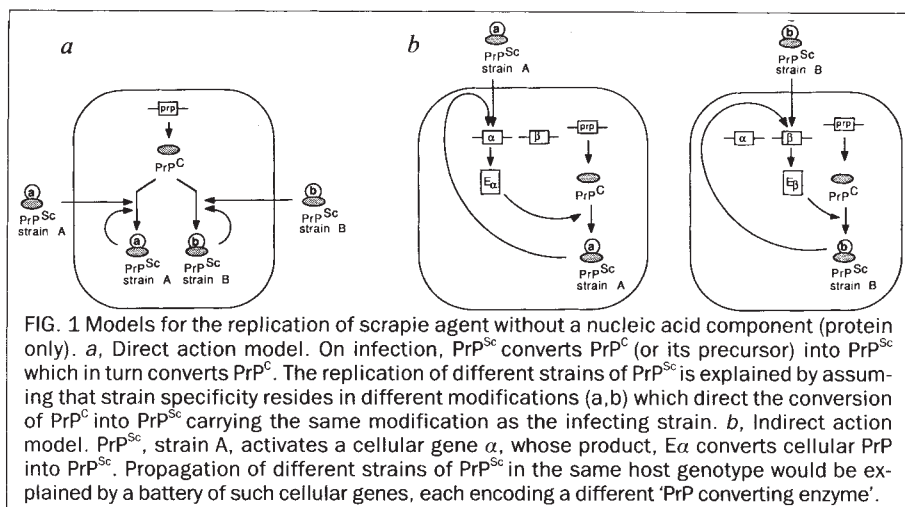
Observations of this type could provide compelling evidence that perceptual mechanisms of the brain do engage in the analysis of brain rhythms. The suggestion that coherent oscillations in activity identify the members of subsets of a large neuronal population may, however, still be an important one, even if these subsets are not closely tied to perception. Exploring the rhythms of the brain, revered by the pioneers of electroencephalography but now mostly dismissed as irrelevant to neural information processing, may even come back into fashion. □

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and nucleolytic agents but sensitive to procedures that modify or destroy proteins, it is unlikely to be caused by a conventional virus, plasmid or viroid. The unusual resistance of the infective agent to ultraviolet and ionizing radiation led not only to the conclusion that the mass of the agent is as low as 55,000 (55K) but also that it is devoid of nucleic acid altogether⁴.

Scrapie infectivity was purified several thousandfold from infected brain extracts by a procedure involving proteolysis under conditions where much of the protein but not infectivity is destroyed⁵. The resulting preparations contain a protein of relative molecular mass 27–30K, designated PrP 27–30, as the major component; although they are not free of nucleic acids⁵, no scrapie-specific polynucleotide has yet been identified in them, particularly no PrP-encoding sequence⁶. Surprisingly, PrP turned out to be a host-specified protein, encoded by a single exon of a unique host gene⁷ and to be expressed both in normal and diseased animals⁶. Brains of scrapie-infected hamsters contain two forms of PrP: the scrapie (PrP^{Sc}) and cellular (PrP^C) isoforms. Both proteins have a mass of 33–35 K, but they have different physical properties. PrP^C is anchored to the cell surface by a glycosyl phosphatidylinositol linkage⁸ and can be solubilized with ionic detergents or phospholipase C, whereas PrP^{Sc} cannot. PrP^C disappears under proteolytic conditions while PrP^{Sc} loses only an amino-terminal peptide to yield a protein of mass 27–30K called PrP 27–30. This is why PrP 27–30 is recovered from scrapie-infected but not from normal brains.

So far, no differences in the primary structure of PrP^C and PrP^{Sc} have been detected^{6, 8–10}, nor have any differences



syndrome is a very rare transmissible disease, familial occurrence could have been interpreted as being due to pre- or perinatal infection with an unknown agent. The new linkage data show that a genetic component is essential for acquisition or manifestation of the disease.

A more fascinating consideration is that if the two families that suffer the syndrome are, as suspected, unrelated, and the identical PrP mutations arose independently, the amino-acid substitution would not only represent a genetic marker but also play an essential role in pathogenesis. This interpretation is strongly supported by an impending report describing two unrelated Japanese families with the ataxic syndrome that show the same proline-to-leucine substitution¹⁸. It could be argued in the 'protein only' hypothesis that the amino-acid replacement greatly increases the probability of spontaneous conversion of PrP^C to (an as yet putative) PrP^{GSS} which would then behave like a transmissible agent (it will be interesting to learn whether in these heterozygous patients PrP^{GSS} contains only the protein with the amino-acid substitution). One might further argue that in sporadic cases of Gerstmann-Sträussler syndrome and Creutzfeldt-Jacob disease a somatic mutation gives rise to a variant PrP with enhanced capacity to convert into PrP^{GSS} or PrP^{CJD}.

Although the new results² tip the scales in favour of the protein only hypothesis, they do not exclude the possibility that a nucleic acid is part of the prion. Taking into account the occurrence of sporadic cases of the diseases, such a nucleic acid or prion would have to be widespread in the population, but with low penetrance in the absence of predisposing PrP mutations. Ultimately, the question as to the nature of the prion should be resolved by generating infectivity *in vitro*, either from pure, non-infectious PrP^C, from a nucleic acid, or from a combination of the two. □

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been found between PrP genes or messenger RNAs from normal and infected brains with respect to structure or copy number. The physical differences between the two proteins are therefore attributed to a post-translational modification. It is known that PrP expressed in uninfected cells from the cloned PrP gene does not cause scrapie¹¹.

Strains of scrapie prions differing with respect to incubation time and brain vacuolation pattern^{12,13} can be passaged many times in the same inbred mouse strain and retain their characteristic properties. Interconversion of certain scrapie strains occurs reproducibly, particularly on transfer from one host to another¹³. Thus, scrapie prions have heritable properties. Incubation time is, however, also determined by a host gene designated *sinc* or *prn-i*^{12,14}; remarkably, the *prn-i* gene is closely linked or perhaps identical with the PrP gene^{14,15}. Mice with short and long incubation times have different amino acids in positions 108 and 189 of the PrP protein, suggesting that the structure of host PrP affects incubation time.

There are two main hypotheses for the

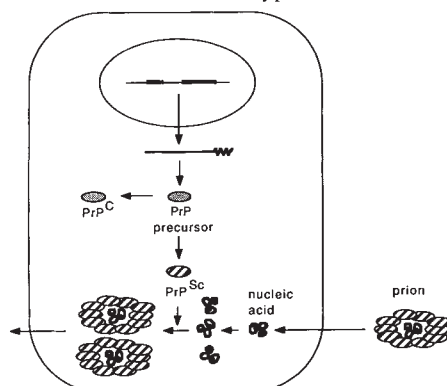


FIG. 2 Model for the replication of scrapie agent containing PrP^{Sc} and a nucleic acid. A nucleic acid associated with host-encoded PrP^{Sc} infects the cell. After replication of the nucleic acid, infectious particles are assembled from nucleic acid and PrP. Conversion of PrP^C to PrP^{Sc} is caused by the association with the nucleic acid, or (as here) PrP^C or its precursor may first be converted to PrP^{Sc}.

nature of the prion. First, the 'protein only' hypothesis proposes that the prion is a protein or protein derivative, devoid of nucleic acid^{1,4}, for which PrP^{Sc} is the most likely candidate. The primary PrP translation product would have to be converted into the modified, infectious form in the PrP^{Sc}-infected cell. This conversion might be directly catalysed by PrP^{Sc} (ref. 16). Each scrapie strain would be represented by a different variant of PrP^{Sc} which modifies one precursor into a product resembling itself (Fig. 1a). Strain conversion on passaging in a different host could be accounted for by polymorphic variants of the resident PrP gene. In a more complex model, conversion of PrP^C to PrP^{Sc} would be catalysed by a host-encoded 'converting enzyme' induced by PrP^{Sc}. The variety of scrapie species would be explained by a battery of distinct converting enzymes, each activated by the cognate strain of PrP^{Sc} (Fig. 1b).

The second, 'nucleoprotein' hypothesis, postulates that the prion consists of a small nucleic acid and host-encoded protein^{3,17} for which PrP^{Sc} is the most likely candidate (Fig. 2)⁵. Strain differences are ascribed to variations in the nucleic acid, just as in the case of viroid RNA.

How do the new data on Gerstmann-Sträussler syndrome reported in this issue² affect our knowledge of prion diseases? In two apparently unrelated families, one in the United States and one in the United Kingdom, Prusiner and colleagues find that an ataxic form of the syndrome is linked to a change in codon 102 in one of the PrP alleles from proline to leucine. Prusiner and colleagues do not find this mutation in 100 normal individuals or in 15 individuals suffering from other forms of inherited or sporadic prion diseases. Using the data from both pedigrees, assuming dominant inheritance and making certain assumptions regarding age-dependent penetrance as well as disease and marker frequencies, the authors calculated a lod score of about 3.3, which is highly significant evidence for linkage. As Gerstmann-Sträussler

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Observations beyond the limit

Demosthenes Kazanas

CYGNUS X-3 is one of the brightest and most extraordinary objects in our Galaxy. It was first discovered as an X-ray source in 1966 and identified as the source of a giant radio outburst in 1972. Subsequent observations of Cyg X-3 have heralded the extension of the energy range for astronomy first to teraelectron volts (1 TeV = 10^{12} eV) and then to petaelectron volts (1 PeV = 10^{15} eV). Now Cassiday *et al.*¹, using the 'Fly's Eye' detector in Utah, have observed Cyg X-3 at higher energies still — exaelectron volts (1 EeV = 10^{18} eV) and above. The authors estimate the probability that the excess of cosmic rays at this energy observed from the direction of Cyg X-3 could occur by chance in a uniform distribution of showers is only 6×10^{-4} .

Collisions of high-energy primary cosmic rays in the upper atmosphere initiate showers of secondary particles which can be observed by astronomers. The Fly's Eye detector used by Cassiday *et al.*¹ maps the nitrogen fluorescence excited in the atmosphere by such showers and has a coverage of 2π steradians. Since November 1986, the operation of two such detectors has permitted three-dimensional reconstructions of the showers. The TeV cosmic rays are observed via the Cerenkov (electromagnetic shock wave) radiation emitted by charged secondaries. And PeV rays initiate showers of electrons (and muons) observed directly by detectors on the ground. Each technique has a resolution of 1° , so that point sources can be identified.

Interest and scepticism

Since its introduction in the early 1980s the field of astronomy at energies above 1 TeV has been viewed with both interest and scepticism². The interest stems from the apparently counterintuitive fact that a large fraction (a tenth) of the luminosity of galactic accreting objects is emitted at energies above 1 TeV — well above the potential energy at the surface of even a neutron star — and in some cases up to 1,000–10,000 TeV. The scepticism arises from the rather low statistical significance of the observations owing to the small number of particles arriving from these sources at these energies: at such high energies, only a few particles are needed to contribute significantly to the total luminosity of an object. It was hoped that new larger detectors would settle the difficulties with statistical significance.

Unfortunately, observations of Cyg X-3 with one of these detectors reported recently³ provide only upper limits to the flux at PeV energies, and the observers arrived at the unsatisfactory conclusion

that PeV emission ought to be episodic. This conclusion is nonetheless strengthened by observations of another source, Her X-1, an X-ray pulsar (period 1.2378 s), which has also been detected in the TeV and PeV range^{4,5}. These observations indicate that at energies of 1 TeV or more, the signal from this source appears in bursts lasting anything from a few minutes to a hundred. A search for periodicities in these bursts indicates a period of 1.2357 s — an intriguing result, as this is significantly different from the period of the X-rays. To compound the puzzles, the muon content in the PeV showers is inconsistent with the assumption that they are initiated by photons (if they were, there should have been ten times fewer muons).

Instead, the muon content is consistent with that expected from hadron-initiated showers. But any such particles, if electrically charged, would be disturbed while traversing the turbulent interstellar magnetic field, so that the observed directional and temporal coherence of the showers could not have been preserved. And there are no known stable neutral particles that could fit the description.

Amid all these puzzles and problems there has been a significant recent advance which may put the whole field on a much firmer ground⁶: the detection of a statistically highly significant (nine-standard-deviation), constant signal of 1-TeV photons from the Crab nebula (background showers initiated by cosmic-ray hadrons were identified and subtracted). This is by far the most significant very-high-energy detection and was achieved by imaging the showers' Cerenkov radiation, a technique which allows the rejection of 98 per cent of the background events. The importance of this result lies in that it provides a weak (10^{34} erg s⁻¹) but steady 'standard candle' against which future experiments will be calibrated.

What can be learned from all these observations? First, a large fraction of the accretion energy in many X-ray sources is converted (albeit sporadically) into relativistic protons with energies greater than 1 TeV (it is generally agreed that it is too difficult to accelerate electrons to these energies) which subsequently produce the observed high-energy radiation through nuclear collisions. Several models for these sources have been proposed (see ref. 8 for a review) which, however, are rather unconstrained owing to the scarcity of photons and lack of repeatability of the observations. Their most important feature is the estimate of the maximum energy expected (about 10^{16} – 10^{17} eV) by the acceleration mechanism favoured by each model (shock acceleration near the

compact object or large-scale static electric fields).

Given their simplicity, the agreement of these models with the observations in the 10^{15} – 10^{16} -eV range is rather remarkable. With the maximum particle energy already set by the models, it would seem that the new observations of Cassiday *et al.*¹ are in direct conflict with theory, especially if the observed particles are photons, which would have to be generated at the source by particles of energy 10^{19} eV. Cassiday *et al.* point out, however, that the observed showers could be due to neutrons. (The Fly's Eye cannot distinguish between photon- and neutron-induced showers; also neutrons, although unstable, can get to Earth at these energies without much attenuation from decay.) Indeed, Ellison and I have proposed⁷ that neutrons are copiously emitted from these objects and could be observed on Earth.

High-energy tail

Furthermore, if the spectrum of the protons accelerated in the source decreases exponentially above the energy of maximum intensity suggested by the current models (10^{17} eV), the energy flux at 1 EeV should be $\exp(10) \approx 10^4$ times smaller than that at lower energies, in rough agreement with the observations obtained with the Fly's Eye. These observations might therefore probe the high-energy end of the particle distribution that produces the radiation at PeV energies.

As for the muon content of the showers, the situation remains confused. An obvious solution is an unknown strongly interacting particle; more appealing, to my taste, is the recent proposal¹⁰ that the quantum-chromodynamic structure of the photon causes an increase in the photo-production cross-section for particle collisions at energies of 0.5 TeV in the centre of mass. At these energies, the photons can interact with nuclei in the atmosphere by the nuclear strong force, a process which may compete with the electromagnetic ones considered so far in the shower development and enhance the muon content. These showers might hence be pointing to new physics at these energies, to be investigated with further observations and detailed modelling. □

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Fifth force remains elusive

Christopher Stubbs

COULD there exist a fundamental interaction that has until recently escaped detection? This notion was addressed at a recent workshop* where participants assessed the current status of the 'fifth force'. The geophysical evidence for a violation of the inverse-square law of gravitation, one way such a force could be manifested, emerged weakened from the meeting, and the results reported from experiments investigating the universality of free fall were in accord with Newton's hypothesis. Nevertheless there remain some tantalizing indications that a new force exists, and given the advances made in the past year the consensus was that experimental effort should continue.

The suggestion 3 years ago¹ of a previously unobserved fifth force prompted a flurry of experiments designed to test the idea. Two types have so far been performed: tests of the weak-equivalence principle comparing the forces exerted on two different substances by some attractor; and geophysical tests of the inverse-square law that seek deviations from the newtonian prediction in the vertical gravity profile. Results are typically expressed in terms of a 'Yukawa' modification to newtonian gravity

$$V_s = \alpha \left(\frac{q_s}{\mu} \right) \left(\frac{q_s}{\mu} \right) G \frac{M_1 M_2}{r} e^{-r/\lambda}$$

where λ and α are the range and dimensionless strength of the interaction and $GM_1 M_2 / r$ is the familiar newtonian expression relating the gravitational potential between two masses M_1 and M_2 to their separation r and the gravitational constant G .

The relevant 'charge' per atomic mass unit q_s/μ is generally assumed to be some linear combination of B/μ and L/μ , with B and L baryon (nucleon) and lepton (electron) number for the material, respectively. Experiments testing the equivalence principle compare the accelerations of materials presumed to differ in q_s/μ ; and the geophysical measurements seek the effects of the exponential term in the potential. The original fifth-force hypothesis favoured $q_s = B$, $\alpha \approx 0.01$ and $10 \text{ m} < \lambda < 1,000 \text{ m}$ (see the discussion by De Rújula in News and Views²).

Statistically, the most significant geophysical evidence for a departure from newtonian gravity comes from measurements of $g(z)$, the vertical profile, up a 600 m television transmission tower³. It is claimed that these measurements differ from the newtonian prediction calculated from an array of surface gravity measure-

ments. These tower data indicate an additional attractive coupling rather than the repulsive force consistent with earlier $g(z)$ observations made in a mineshaft in Australia⁴. However, D. Bartlett and W. Tew (Univ. Colorado) question whether local topography was properly taken into account in the tower experiment, prompting the experimenters carefully to re-examine their data (D. Eckhardt, USAF Geophysical Lab.). In fact the surface gravity measurements were obtained at locations whose mean elevation differed from that of the actual terrain. Correcting for this has reduced the maximum discrepancy between the modelled and observed $g(z)$ from $550 \mu\text{gal}$ to $350 \mu\text{gal}$ ($1 \text{ gal} = 1 \text{ cm s}^{-2}$). Preliminary results from two other tower experiments (T. Niebauer and C. Speake, Univ. Colorado; P. Kasameyer and J. Thomas, Lawrence Livermore National Laboratory) appear so far to support conventional physics. The task of computing newtonian gravity properly is now generally recognized as the most delicate aspect of these experiments, particularly when assigning uncertainties to the prediction.

Gravity measurements performed down the 2,000-m Dye 3 borehole in Greenland were extensively reported last autumn when the preliminary analysis seemed to indicate a departure from the inverse-square law. The experimenters now report that although the borehole $g(z)$ data (discussed by M. Gorman, Cambridge Univ. and R. Hughes, Los Alamos National Laboratory) disagree with the group's initial newtonian prediction, this discrepancy could well be the result of an intrusion of higher-density

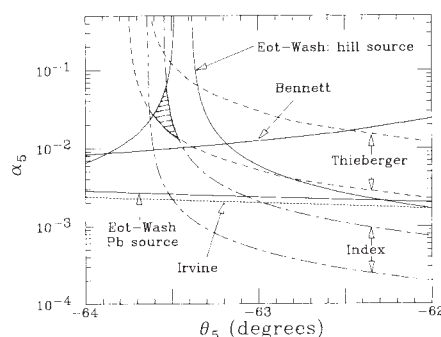


FIG. 1 2σ Constraints on a fifth force coupled to Isospin, for $\lambda = 100 \text{ m}$. Allowed values of α ($\lambda = 100 \text{ m}$) are plotted as a function of θ_s , where $q_s = B \cos \theta_s + L \sin \theta_s$. A coupling to 'isospin' corresponds to $\theta_s = -63.4^\circ$. The shaded region shows the area of agreement between the composition-dependence experiments. However, this is now of dubious significance given the lead-attractor results reported by the Eöt-Wash¹³ and Irvine (Newman) groups. A slightly less stringent constraint is shown by the lock experiment of Bennett¹⁴.

material ($\Delta \rho \approx 0.3 \text{ g cm}^{-3}$) in the rock below the ice cap. The likelihood of such an intrusion is much debated, even by members of the team itself. The group used an optimization code to determine the mass distribution of minimum $\Delta \rho$ that could account for the $g(z)$ data while remaining consistent with surface gravity observations. Applying a similar analysis to the gravity data available from the tower and mineshaft measurements (some are proprietary) has led to the claim (R. Parker, Scripps Institution of Oceanography) that the observed $g(z)$ discrepancies in those experiments can also be attributed to an appropriate local distribution of higher-density material. This suggestion is currently being explored by the experimental teams.

Among the experiments testing the equivalence principle, two^{5,6} have produced results consistent with a new material-dependent interaction, but others⁷⁻⁹ saw no such effects. These initial composition-dependence results were contradictory when interpreted in terms of $q_s = B$ (see News and Views articles published at the time^{10,11}). Efforts have recently been focused on approaches for reconciling the positive observations with the null results, and on extending the search by using apparatus with increased sensitivity.

The suggestion^{6,12} that a coupling to $q_s = B - 2L$ (or isospin) could effect a reconciliation of the composition-dependence results has been closely inspected. This isospin picture amounts to supposing that the neutron and proton are endowed with 'charges' of opposite sign but equal magnitude. Because the Earth's crust (the attractor in the early experiments) is composed mainly of materials (silicon and oxygen) with equal abundances of neutrons and protons, the net q_s of the various experimental sites would depend on the detailed chemical composition of the local rock.

This hypothesis is readily tested by using either a proton-rich or a neutron-rich substance as the attractor, instead of terrestrial material. One such experiment has been performed by our Eöt-Wash group at the University of Washington, by comparing the interactions of beryllium and aluminium with 1.3 metric tons of lead. We have just published¹³ a null result that excludes (at 2 standard deviations) the isospin picture for all scale lengths over which the positive results have been interpreted ($10 \text{ m} < \lambda < 1,000 \text{ m}$).

Preliminary results presented at the meeting from a similar experiment (R. Newman and P. Nelson, Univ. California, Irvine) yielded a comparable upper bound. Another interesting experiment¹⁴ used a torsion balance near a lock on the Snake River in the United States. The quantity of water acting on the balance was varied by the periodic filling and

* XXIV Rencontres de Moriond: Tests of Fundamental Laws in Physics Les Arcs, Savoie, France, 21–28 January 1989.

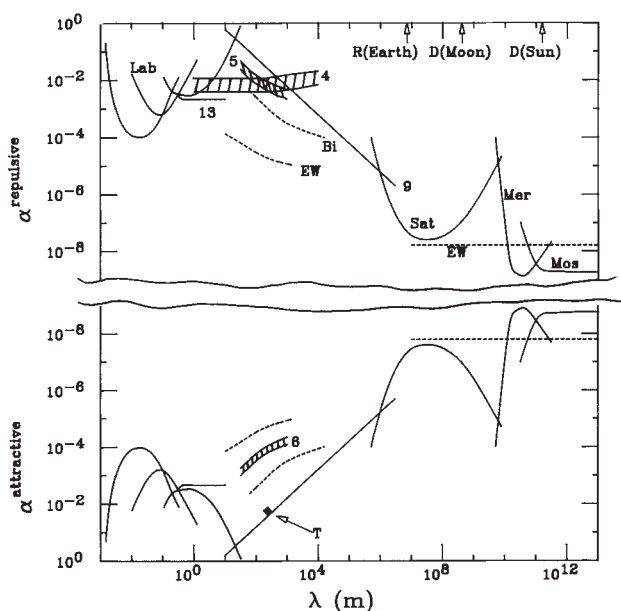


FIG. 2 Constraints on the strength (relative to G) of a Yukawa interaction coupling to B , for ranges λ between 1 mm and 10^{13} m. The positive results are shown as the shaded regions labelled 4, 5 and 6, in accord with the references. T denotes the best single-Yukawa fit to the revised tower data of Eckhardt *et al.*, who are still in the process of determining uncertainties. For clarity only the most stringent of the many null results are shown, which preclude values of α greater than the corresponding constraint curves. Bounds that predate the fifth force controversy (see ref. 2) arise from laboratory $1/r^2$ tests (Lab), from comparisons of the orbits of manmade satellites with that of the moon (Sat), from the precession of Mercury (Mer), and from the equivalence principle experiment performed in Moscow by Braginskii and Panov (Mos). Line 9 is from the freefall experiment of Niebauer *et al.*⁹, and 13 from the Eöt–Wash torsion balance experiment¹³. The dashed segments EW and Bi show the results reported at Moriond by the Eöt–Wash group and by Bizzeti *et al.*

emptying of the lock. The data analysis sought any deflection of the balance that was correlated with water level, and none was found. Results from these isospin experiments are presented in Fig. 1.

New data were presented from two terrestrial-attractor equivalence-principle experiments. A floating-sphere experiment reminiscent of the earlier one by Thieberger⁵ was performed on a large-scale escarpment in Italy, by P. G. Bizzeti and collaborators (Univ. Firenze). After a careful search for systematic errors, the experimenters concluded that there was no discernible difference between the hillside's action on the submerged nylon sphere and its influence on the surrounding water. The upper limit on the horizontal acceleration difference of the two materials is 2.4×10^{-9} gal. This null result is particularly significant for $1.5 \text{ km} < \lambda < 16 \text{ km}$.

My colleague E. Adelberger presented preliminary hillside results that our team has obtained with a much improved version of our torsion balance. We observe no acceleration difference between beryllium and aluminium at the level of 4×10^{-11} gal, establishing the most stringent limit thus far on a composition-

dependent interaction for $10 \text{ m} < \lambda < 1.5 \text{ km}$. The current experimental limits on $\alpha(\lambda)$ are shown in Fig. 2, with the various results interpreted in terms of $q_5 = B$.

The initial fifth-force hypothesis of an intermediate-range interaction with $q_5 = B$ is not consistent with experiment. In fact no physically reasonable phenomenological model advanced to date can account for all the data, which is not too surprising given the apparent contradiction in the results.

Applying Occam's razor to the positive observations leads to the suspicion that there are unappreciated sources of systematic error in one or more of these delicate and demanding experiments. It should nevertheless be remembered that only one of the positive observations need be convincingly verified for there to be evidence of new physics. Theorists have shown that a feeble intermediate-range interaction could come about in various ways,

and could be readily accommodated in several pictures of the world beyond the standard model. The possibility that these experiments may provide a window into physics that is well outside the reach of any existing or planned accelerator is sufficient motivation to continue the search.

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Digital puppetry

TELEVISION transmission is absurdly inefficient. A TV picture is retransmitted in its entirety about 30 times a second, taking up about 6 megahertz of bandwidth; the new high-definition format will need 30 megahertz. Yet the incremental optical changes could probably fit comfortably into a mere few kilohertz.

So Daedalus's new Electronic Theatre TV system makes no attempt to transmit a picture. Like the human perceptual system, it codes the scene as a physical model: a 'set' or 'backdrop' with a specified appearance, in front of which a 'cast' of objects (furniture, people, cars) stand or move in a specified sequence of positions. From this description, together with defined lighting and camera position, an image-reconstruction routine in the receiver works out what the scene must look like, and displays it.

Transmission bandwidth is utterly minimized. The transmitter downloads its backdrop and cast of characters only once per scene, like a word processor downloading to a printer the font in which a document is to be printed. Thereafter 'stage directions', simple sequences of spatial coordinates and details of changes of appearance, suffice to update the image. Massive real-time computing effort is needed both at transmitter and receiver; indeed, the scheme stretches existing technology to its limits. But since computing speed and power are increasing at a hectic pace, while communication bandwidth (especially geostationary-satellite bandwidth) is ever more scarce and costly, Electronic Theatre should soon be commercially feasible.

The new system will change production methods as well as transmission. Producers and playwrights will increasingly by-pass the camera and studio altogether. They will write a show or a play directly as a script of stage directions — calling up backdrops and casts from stock digitized libraries, manoeuvring them in program form, and studying the resulting visual effect. Viewers, too, will gain. A television set will be able to substitute a local 'font' of items and characters into any broadcast: turning a global soap-opera, for example, into a national, or even a family, story. The chilling uniformity threatened by global TV will be mitigated. Mischievous viewers who want their news read by Mr Gorbachev in drag, say, will also have their wish. And like the live theatre itself, an Electronic Theatre production can be endlessly renewed and re-interpreted. Old dramas will be re-set in different locations, transferred to nostalgic eras, or equipped with exotic characters. Electronic Theatre will not only release bandwidth for thousands of new channels: it will provide the semblance of variety to fill them. David Jones

Are comet spins primordial?

SIR—Ferrin¹ contends that several comets, including Halley, rotate at rates determined during their primordial accretion. He bases this on the fact that comets satisfy $L/M \approx M^{2/3}$ (L is spin angular momentum and M is mass), a relation approximately fulfilled by virtually all other solar system members. From this, he infers very low densities ρ for several comets.

Here, I argue that (1) the L/M relation indicates only that spin periods T of most Solar System members are about the same; (2) deviations from the constant-spin rule in other small Solar System bodies can be attributed to current processes; (3) the spin rates of asteroids and comets have been significantly altered by collisions and outgassing, respectively, and are thus not primordial; and (4) the L/M relation, although perhaps useful as a crude guide, is not a reliable indicator of density for a specific object.

For a homogeneous rotating sphere, $L/M = 0.97M^{2/3}/(T\rho^{2/3})$; a similar expression is known for triaxial ellipsoids^{1,2}. Thus, if density differences between different celestial bodies can be ignored, the observation that $L/M \approx M^{2/3}$ implies that rotation periods of Solar System members are roughly the same, ~ 8 h, as originally noted for 70 asteroids and most planets³.

Rotation periods of cometary nuclei are poorly known because the classical halo method⁴ and precession model⁴ are of unproved accuracy. Except for Halley's case, the only reliable values come from recent CCD observations of three cometary nuclei⁵ whose rotation periods are 12–22 h, significantly shorter than the 30–50 h periods Ferrin used. As cometary data are so scant, one might ask whether the spins of other small Solar System objects say anything about their beginnings.

The spins of almost 500 asteroids are now known⁶. All but 1–2% have periods within a factor of 3 of the mean, $\langle T \rangle \sim 9$ –10 h, similar to most planets, a few nontidally despun satellites, and probably not too far from cometary periods. Furthermore, the considerable variations in asteroidal spins with their size⁶ belies Ferrin's claim that celestial rotations are fixed primordially. The data show that: (1) large asteroids (radius $R \geq 62.5$ km) have $\langle T \rangle \approx 7.5$ h and a Maxwellian distribution of spin rates, implying that collisions are important; (2) minor planets with $R \approx 40$ –50 km spin more slowly ($\langle T \rangle \approx 11$ h), a fact generally ascribed to angular momentum drain⁷; (3) the smallest asteroids ($R < 15$ km) rotate faster in the mean ($\langle T \rangle \approx 8.5$ h) but also have a wider dispersion, especially showing an excess of

slow rotators; they do not obey a Maxwellian distribution, perhaps indicating their birth in catastrophic disruption events⁸; (4) spin characteristics also vary with asteroid taxonomy, with M-asteroids, presumed to be metal-rich and relatively dense, rotating fastest⁶.

Small asteroids and comets have had significant angular momentum imparted to them over the aeons. For the former, simple particle-in-a-box calculations⁸ find frequent mutual collisions, both erosive and catastrophic, that re-set rotation rates, especially those of the tiniest asteroids. In contrast, comets rarely encounter a compatriot in the spacious Oort cloud nor often meet other projectiles during their brief sojourns within the inner Solar System, so collisions do not appreciably alter their angular momentum. Asymmetric outgassing may however induce comets to twirl faster or slower. The same gas jets that cause the non-gravitational accelerations of cometary orbits produce torques capable of modifying spins. Taking the tangential outgassing reaction force to be 10^{-6} of solar gravity⁹ and, for a lower bound on de-spin time, considering this force to have a moment arm R , spin rates are found to change dramatically over only a few orbits. A similarly swift de-spinning comes from estimating the angular momentum carried away by the sublimated gas. Because expansion velocities in jets are a few hundred m s⁻¹ whereas typical surface speeds are ~ 1 m s⁻¹, much less than one per cent of the comet's mass, if lost tangentially to the surface, could remove the body's spin angular momentum. Such a mass is expelled in just a few passes by the Sun.

The rough rule that celestial objects — whether asteroids, comets or pulsars — spin faster when they are denser simply manifests the greater ability of compact objects to hold themselves together as they are spun up. Equatorial surface layers on solid spheres become centrifugally unbound once $T \leq 3.3$ (ρ/g cm⁻³)^{-1/2} h (refs 2,3,5) while debris on the most distant tips of triaxial bodies will be lost at even slower rates². Loosely bound agglomerates (rubble piles), such as sometimes proposed for comets¹¹, flow and elongate at spin periods less than ~ 6 (ρ/g cm⁻³)^{-1/2} h (ref. 12).

Whether one looks at Solar System rotation data in terms of a constant spin-rate law or some universal L/M plot, one should not take the results too literally. While appearing profound, L/M relations, which also seem to apply to other classes of astronomical objects, have been dismissed¹³ because they merely reflect reasonable upper and lower limits on orbital and/or spin rates, much as I maintain above. Furthermore, data that look so

remarkable on log-log plots over many orders of magnitude in the mass actually show considerable scatter of individual points. As an extreme example, 288 Glauke, an 18.5-km S-object, takes 1,150 h to rotate while the comparable asteroid 321 Florentina spins in 2.87 h; are we to conclude that their densities differ by a factor of 8,000?

These arguments show that the observed rotations of comets and small asteroids are not primordial. Instead, their spins probably result from a partly delimited evolution. Torques, which act sporadically to spin these bodies up or down, cannot produce very rapid rotations because of the centrifugal breakup limit; the end result is that spins are always found somewhere between zero and this limit. Clearly for asteroids, rotation rates do hint at processes of angular momentum transfer. But too little is now known about cometary rotation for cometary origins or properties to be valuably constrained.

On the other hand, it may be that, as the Duchess in *Alice in Wonderland* believed, "If everybody minded their own business, the world would go round a great deal faster than it does."

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On *Prochlorothrix*

SIR—In his News and Views article¹, David Penny noted the conflicting results of two comparative biochemical studies of *Prochlorothrix* and other photosynthetic prokaryotes — a study by Turner *et al.*² on the 16S ribosomal RNA subunit and a study by Morden and Golden³ on psbA, a photosystem II-associated protein. Turner *et al.* place *Prochlorothrix* close to *Synechococcus* in a separate line of descent from green chloroplasts, whereas Morden and Golden suggest a closer relationship between green chloroplasts and *Prochlorothrix*. Although these results are inconsistent, it should be noted that both studies^{2,3} are a preliminary part of the effort to place the Prochlorophytes on the basis of the limited amount of available

experimental data. The lack of information about possible polymorphisms in natural populations, for example, is an issue which needs to be addressed by further study.

We would like to add that there is an important body of structural data which should be considered before lineage maps are drawn on the sole basis of a few nucleic-acid sequences. The photosynthetic membranes of green chloroplasts have a characteristic, well-studied structural organization, which includes a biochemical segregation of photosystems and other components into stacked (granal) and non-stacked (stromal) membrane regions⁵. Studies on *Prochloron* carried out by others⁶ as well as our recent study⁷ on *Prochlorothrix* indicate that surprisingly many of the structural details of photosynthetic membrane architecture are identical in green chloroplasts and prochlorophytes. In *Prochlorothrix*, these include membrane appression, an asymmetrical distribution of intramembrane complexes between stacked and non-stacked membranes, the sizes and shapes of particles in membrane fracture faces, and the existence of a tetrameric complex on the membrane inner surface which (in green chloroplasts⁸, at least) is associated with oxygen evolution.

The wealth of structural similarity between prochlorophyte and green chloroplast photosynthetic membranes suggests a much closer relationship between *Prochlorothrix* and *Synechococcus*. It also casts doubt on the ease with which one can suggest, as do Turner *et al.*², that "the acquisition of the ability to synthesize chlorophyll *b* would not seem to be a significant biochemical change". Although the chemical differences between chlorophylls *a* and *b* are indeed minor, the similarities between prochlorophytes and green chloroplasts run much deeper than the aldehyde group of chlorophyll *b*. It is a major pattern of membrane architecture that they have in common, not just the possession of the same chemically modified form of chlorophyll *a*. The independent evolution, in two separate lines of descent, of nearly identical patterns of membrane appression, architecture and photosystem segregation seems, to us, unlikely.

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Sympatric pest

SIR—Three recent letters^{1–3} and an accompanying News and Views article⁴ concern the fascinating story of how, in the past 200 years, the native American fly *Rhagoletis pomonella* has apparently moved from its native hawthorne host to become an important pest of commercial apple crops. The assumption is made in all four papers that the clear genetic features characterizing the populations breeding on apples have arisen both sympatrically and very recently, evolving from a resident American population that originally infested only the native hawthorne. But an alternative, if less interesting, explanation for the genetic origin of this pest is also possible.

Before the introduction of the apple, there may have been two or more genetically distinct races of *R. pomonella*, adapted to different hawthorne species or possibly to an endemic native crabapple, such as *Pyrus coronaria*. One such population could have been preadapted for the exploitation of the commercial apple and could then have multiplied, with only minor genetic change, into the abundant pest as we know it today. The rapid sympatric evolution of a host race would not then be a required explanation; the transfer from hawthorne to apple could have been simple colonization of a newly available host.

The hawthorne genus (*Crataegus*) in the north central United States and adjacent regions of Canada consists of over a hundred taxonomically diverse and confusing species belonging to 19 series (species groups)⁵. There are additional varieties, hybrids and/or apomicts. At least 40 recognized species of *Crataegus* occur in the Michigan-Illinois region and more than 20 others are found in the limestone refugia to the south. These latter are ancient highlands that go back to the Permian. They were never covered by glaciers, nor were they ever flooded like the coastal plain or the inland sea. They were the source of most of the colonizers of the recently glaciated areas of Ohio, Michigan, Indiana and Illinois to the north⁶. *R. pomonella* has surely coexisted with many of this truly colossal number of hawthorne species for thousands — or even millions of years, moving south with the plants as the glaciers advanced and then moving back north as the ice retreated.

Must it be assumed that there were no

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host races of *R. pomonella* formed during all this time but that a new one was formed in the past 200 years? Sympatric origin of host races is by no means a trivial matter, either for practical pest management or for evolutionary genetics. Accordingly, before accepting the evolution of a distinct host race that arose sympatrically in historic times, further data on the populations that breed on hawthornes must be sought.

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Our ancestors

SIR—Hominids and the African apes form a well-defined clade, but there is disagreement on the initial divergence of that clade. Most molecular evidence favours the gorilla as the first to diverge, whereas most morphological evidence puts hominids there. The second alternative is mildly supported by evidence from chromosomes¹. The evidence in each direction is strong and is based on independent and internally complex data, which have been examined by Andrews^{2,3} in an excellent review. Later work merely accentuates the problem, which exists primarily because it is taken as axiomatic that the point of divergence was the same for all characters. This need not, however, have been the case.

Perhaps all three groups diverged from the same species, which may have been geographically variable. If so, reassortment of the results of local evolution could produce conflicting apparent phylogenies if one looks at only part of the evidence. As a simple example, the diverse aspects of knuckle-walking and thinner enamel may have begun to evolve in a proto-gorilla subspecies and then been transferred to proto-chimpanzees after the latter mostly separated from proto-hominids.

Such a resolution of the problem is a bit awkward, but on existing evidence it is not as awkward as the alternatives⁴. It has no bearing on classification except to a cladist. Accumulation of further evidence like that now available would support the tritomy (or autotomy?). In contrast, establishment of a long initial divergence time, by fossil or molecular evidence, would make it implausible.

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Great expectations

Hans Primas

Beyond the Atom: The Philosophical Thought of Wolfgang Pauli. By K.V. Laurikainen. Springer-Verlag: 1988. Pp. 234. Pbk DM68, £22.50, \$29.

WOLFGANG Pauli (1900–1958) was a revolutionary genius, a most critical theoretical physicist with profound insight, and a deep thinker. He was an infant prodigy — at the age of 18 he was in full possession of the mathematical and physical knowledge enabling him to do original research on general relativity. At 21 — still a student — he published in the *Encyklopädie der mathematischen Wissenschaften* his masterly review article on relativity theory, which was greatly admired by Einstein himself. Later he became renowned for his fundamental work and brilliant reviews on quantum mechanics and quantum field theory.

Pauli has been called “the living conscience of theoretical physics”, “der fürchterliche Pauli” and “god’s whip” because he attacked every half-truth with pitiless severity. But his criticism was always sound and came to be relied upon. In Pauli’s view, there was no basis for the type of unified field theory pursued by Einstein. But in spite of this and other deep epistemological disagreements, the old Einstein held Pauli in high esteem and called him his spiritual son.

Although Pauli is recognized as one of the leading theoretical physicists of the twentieth century, his long-standing philosophical efforts and penetrating jungian studies are less well known. Only a few of his published articles deal with epistemological problems — the technical papers are remarkably free of philosophical comments. But this state of affairs gives an entirely misleading impression of Pauli’s wide range of philosophical and historical interests, and his far-reaching commitment to jungian thought. Professor Laurikainen’s *Beyond the Atom* — in the main a translation of his Finnish book *Atomien Tuolla Puolen* of 1985 — will, one hopes, change things. The book is based on the extensive but yet unpublished correspondence between Pauli and Markus Fierz, Pauli’s former postdoctoral assistant (1936–1940) who at the time was professor of theoretical physics in Basle.

Pauli took up the psychophysical problem by studying the connection between the complex psychology of Carl Gustav Jung and modern physics. In particular, he investigated the archetypal background of physical concepts, and published a remarkable case study on the genesis of scientific theories in the light of the historical controversy between the trinitarian point of departure of Kepler’s astronomy and Fludd’s quaternarian

alchemical viewpoint. Quaternity is taken to be an expression of all concepts of unbroken totality, both in psychology and in physics. The “quaternarian attitude” (an expression often used by Pauli in his letters) is not easy for a classical scientist to grasp. Pauli and Jung discussed the conjecture that the statistical method of natural science could be in a complementary relationship to synchronicity, in the sense that any statistical description excludes synchronistic phenomena. They proposed that the triad of energy, space-time and causality should be complemented by the synchronicity factor to arrive at a “quaternion”, which stands for the unity of being.

In order to express this idea of an unbroken unity, Pauli was looking for a

new conceptual language between the physical and archetypal domains, a language that was neutral with respect to the distinction between physical and psychological phenomena. He conjectured that the alchemistic attempt to create a unitary psychophysical language failed merely because it dealt with a visible reality, and he hoped that the same programme, referring instead to a deeper invisible reality, could be successful. That is, Pauli was striving for nothing less than a coherent and unified conception of the world, in which natural science would be just a part, and which would allow an understanding of matter and psyche as complementary aspects of the same reality — a reality containing both rational and irrational elements. Because the logical structure of complementarity has become transparent within the mathematical formalism of quantum mechanics, it is not so extraordinary that it is just theoretical physicists who have recognized the road sign and have taken up the investigation of complementary relationships outside natural science.

According to Laurikainen, the Pauli-

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REASONS

Wolfgang Pauli — “striving for nothing less than a coherent and unified conception of the world”.

Fierz letters are the best source for the study of Pauli's philosophical views. In his book, he lets Pauli speak for himself in long quotations from the correspondence, unfortunately without Fierz's answers. Laurikainen's selection of material and his analysis of it unavoidably reflect his own tastes and inclinations, but these limitations cannot lessen my regard for his well-thought-out presentation. The most important message of the book is that if we take Pauli's view seriously, we have to re-evaluate fundamental questions in natural science and ponder about the repression of the irrational in Western culture.

With respect to some details, I found myself repeatedly disagreeing with Laurikainen. Fortunately, this disagreement is mainly about questions of present-day quantum mechanics, which was not an issue in the Pauli-Fierz correspondence.

Laurikainen believes that Pauli would not have agreed with modern foundational research on quantum theory and the current attempts to generalize quantum theory and to apply it to the meso- and macroscopic domain. To my mind it is wrong to pass such a judgement on the basis of the Pauli-Fierz letters.

In the correspondence, Pauli argued that the general trend of Western culture after the seventeenth century has been dangerously one-sided. He considered the cartesian separation of spirit (*res cogitans*) and matter (*res extensa*) to be misconceived and preferred a vision where spirit and matter are considered as two complementary aspects of reality. Pauli was looking for radically new ideas which went far beyond the limits of quantum theory. According to him, the possibility of free choice of the experimental set-up implies that observations acquire the character of the irrational, unique actuality. But he also stressed that "the psychic state of the observer enters the laws of nature of quantum mechanics no more than those of classical physics", and that "the old question whether the psychic state of the observer possibly may influence the external material evolution of Nature, has no place in contemporary physics". We have to acknowledge that there is a lacuna in the reasonings of present-day physics. Pauli tried to open up new paths, but his programme was very ambitious and remains rather vague, so we cannot implement it in our current theoretical framework. As Pauli himself wrote, the problem is that we must not thereby sacrifice the positive values of the trinitarian attitude. I think that Laurikainen sometimes misconstrues Pauli's vision of a unitary theory as being a comment on present-day quantum mechanics.

"The most important message of the book is that if we take Pauli's view seriously, we have to re-evaluate fundamental questions in natural science and ponder about the repression of the irrational in Western culture."

I have one further reservation, also minor. Laurikainen has tried to make Pauli's philosophy understandable to an average academic reader, not only to scientists. Even for the committed reader it is at present the only generally accessible source of Pauli's philosophical correspondence; it is indeed an excellent guide to his views and gives a good first impression of the exchange of ideas with Fierz. But an essay of this nature can never be totally adequate. A full appreciation of Pauli's visions requires a substantial knowledge of quantum mechanics, of the

history of ideas and of archetypal psychology. I suspect that most theoretical physicists, psychologists and philosophers who would like to grasp Pauli's ideas stand in need of much more help than they get here. Even Pauli looked for a helping hand — in an unpublished letter (not quoted

by Laurikainen), he expressed his disappointment about the lack of scientific education of Jung's circle, and his hope of finding a discussion partner with profound psychological insight and a good knowledge of mathematics and the natural sciences. So what we urgently need is a complete edition of Pauli's philosophical correspondence with explanatory notes written by various qualified scholars.

Moreover, there is much more pertinent material in the archives. A wealth of supplementary information can be found in Pauli's correspondence with Carl Gustav Jung, Marie-Louise von Franz and Aniela Jaffé, preserved at the ETH in Zurich. There are also related, unpublished manuscripts by Pauli — such as *Die Vorlesung an die fremden Leute*, or his important *Hintergrundphysik* (which is not lost, contrary to Laurikainen's suspicion) — and there is further correspondence, such as that with Heisenberg in the 1950s. Unfortunately, not all of the curators of these papers have allowed them to be published. It would be an intellectual disgrace if the legal heirs of the Pauli estate are allowed to suppress his visions and dreams (which in Pauli's own judgement unquestionably have an objective content), and publish only those letters that are 'relevant' to the history of physics, or that correspond with prevailing scientific fashions. I hope that the appearance of Laurikainen's ground-breaking and thought-provoking book will encourage the publication of a complete and annotated edition of all Pauli's letters and manuscripts in the near future. □

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The star worm

Paul Sternberg

The Nematode *Caenorhabditis elegans*. Edited by William B. Wood and the Community of *C. elegans* Researchers. Cold Spring Harbor Laboratory: 1988. Pp. 667. \$94.

In 1963, Sydney Brenner wrote in a proposal to the Medical Research Council:

Part of the success of molecular genetics was due to the use of extremely simple organisms which could be handled in large numbers: bacteria and bacterial viruses. ... We should like to attack the problem of cellular development in a similar fashion, choosing the simplest possible differentiated organism and subjecting it to the analytical methods of microbial genetics.

Brenner not only chose the nematode *Caenorhabditis elegans* as the organism, but encouraged a generation of scientists to join him; hence, 25 years on, the publication of this first-rate monograph which reviews the work of some 400 scientists. Besides summarizing the basics of *C. elegans* biology, the book presents in digestible form the main accomplishments — determination of the complete cell lineage and of the 'wiring diagram' of the 302-celled nervous system, and the collection of a genetically mapped set of ordered recombinant DNA clones (a genomic map) which is now over 60 per cent complete.

These projects have been carried out by and large at the MRC Laboratory of Molecular Biology, Mecca for *C. elegans* researchers. What then of the unlucky faithful who are unable to make the pilgrimage but who nonetheless want to participate now that the stage is set for a variety of fundamental studies of developmental, neuro- and molecular biology? By reading the book, one can learn much 'worm lore', as well as gain a sense of the excitement in the field.

A scientist starting work on *C. elegans* a decade ago would have had only a handful of publications to read; now one would confront a staggering number of important papers as well as a vast amount of unpublished data. In these days of chasing problems across diverse phyla, researchers often have to grapple unaided with the intricacies of a novel experimental system. Here is help for such people. For example, the often arcane details of the *C. elegans* field (*unc*, *Dpy*, *B.alapaav*, *HSN* and so on) are made accessible to anyone who wants to get to grips with them. The uniformly high quality of the chapters and the extensive cross-referencing not only reflect careful editing, but the cooperative effort of the authors and editor. A great deal of information is presented, however, and some work on the part of the reader is still required.

The application of molecular genetics to muscle, the nervous system and cell lineage is the hallmark of research on *C. elegans*, as is made evident in several cogent chapters. For example, the ability easily to identify and propagate paralysed mutants defective in a myosin heavy chain made possible the first complete sequence of this polypeptide. Identification of over 100 genes controlling various aspects of development relied on many of the characteristics apparent to Brenner in his choice of the worm, especially its small number of cells and short generation time. Yet although the excitement of recent work on sex determination and germline development is amply conveyed, a chapter devoted to the cuticle and molecular genetics of collagen would have been welcome — several genes defined by morphological and behavioural mutants

encode collagens.

The final third of the book is taken up with helpful appendices. The summary of methods is not a complete laboratory manual, but is a good place to start. A 60-page gene list includes invaluable information for the novice (or expert), such as how easy it is to score the phenotypes of the various mutants.

For any biologist, this monograph can serve as an enticement to study a favourite problem in the worm, or as a source of unanswered questions. One hopes that those so encouraged will retain the co-operative spirit, the high standards and the love of this organism that have characterized the field over the past two decades. □

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The number of the rose

John D. Barrow

Between Quantum and Cosmos: Studies and Essays in Honor of John Archibald Wheeler. Edited by W.H. Zurek, A. van der Merwe and W.A. Miller. *Princeton University Press: 1988. Pp. 623. \$49.50.*

"TIME is God's way of keeping things from happening all at once." Anyone who treasures this amongst their favourite aphorisms is clearly a little out of the ordinary.

John Wheeler is certainly such a person. In honour of his seventy-fifth birthday many of his former students and collaborators cornered six issues of the monthly journal *Foundations of Physics* and filled them with research papers and reviews on subjects to which Wheeler has made fundamental contributions or in which he has a strong interest. Here, these articles are gathered up into a single volume. The result is different both in style and intent from the earlier Wheeler *Festschrift*, *Magic without Magic*, published a decade ago. There are many more contributions, at a much higher technical level, and there are no general biographical articles about Wheeler's scientific career and style of working. Instead there are annotated highlights and characteristic artwork from his most important papers. Yet despite these adornments and the catchy titles of many contributions, this is not a popular book nor even one for scientific outsiders.

The articles fall into five principal categories. The first spans those areas of nuclear physics with a wheelarian pedigree; in particular, the development of the theory of fission (with Niels Bohr), the S-matrix, nuclear models and the role

of nuclear spin-orbit interactions down to the properties of positronium and electrodynamics. The bulk of the book deals with Wheeler's primary interest, gravitation — he was the first to introduce such topical concepts as black holes, wormholes, the Wheeler-de Witt equation of quantum gravity, together with the whole viewpoint of geometrodynamics. The quantum gravity articles in this selection form an interface with those on the interpretation of quantum measurement, where the Everett-Wheeler 'many worlds' interpretation provides the only way of looking at quantum mechanics that makes any sense of the concept of quantum cosmology. The articles by Dicke, d'Espagnat, Ne'eman, Unruh, Braginsky and Khalili, Wothers and Peres all deal with aspects of the quantum measurement problem.

The last and most interesting section of the book contains papers on the concepts of complexity and computation in their most abstract forms. Here there is a long and characteristically insightful discussion of quantum computation by the late Richard Feynman, in which he argues that there are no physical barriers to the reduction of the size of computers to the atomic scale where their mode of operation would become intrinsically quantum mechanical. Landauer and Bennett, two of the foremost contributors to the development of algorithmic complexity as a branch of physics, then each consider the notion of computation and complexity. They show how physics can subject the concept and process of computation to detailed analysis, in order to discover whether the Heisenberg uncertainty principle or the second law of thermodynamics place fundamental limits upon the scope and speed of computation. Bennett provides some discussion of the concept of 'logical depth' as an adjunct to the earlier definition proposed for the complexity of a sequence which used a purely quantita-

tive measure — the shortest program able to generate the sequence — because account must be taken of the difficulty of arriving at the minimal program. Some proposals for achieving this find their way into Bennett's contribution, although the best ideas, which concentrate upon the amount of entropy generated by the program of minimum length, were made after his article was written.

Finally, one should highlight the article by Geroch and Hartle, which describes what it means for a measurable physical quantity to be computable in the sense of Turing, Church and Post. In our mundane experience of physics, non-computable functions have not emerged naturally. It is, though, quite possible for the varieties of differential equation familiar in physics — ordinary differential equations or hyperbolic wave equations — to possess solutions which are non-computable functions of well-posed initial conditions, if one allows the initial behaviour to be not entirely smooth but contain creases and cusps like those found at the point of a cone. Although it is usual for physicists to discount such kinky starting conditions as physically unrealistic, it is not clear that they should do so given the non-continuum nature of classical physics.

Geroch and Hartle discuss a recently discovered example, which arises in quantum cosmology. Here, an observable is found to be equal to a sum of quantities, which must be evaluated for all the compact four-geometries. But the infinite list of these geometries is known to be non-computable. This does not necessarily mean that the observable cannot be computed and predicted — there might be another way that avoids listing the unlistable and so is computable. Nonetheless, it provides an interesting example of how difficult it may prove to answer some of the questions posed in cosmology and particle physics. The ultimate capability of the human brain and of all our computational machines may fall short of that required to rationalize things.

The book makes interesting reading, but it is disappointing in some respects. The articles were originally intended for a journal, and the authors might have written more generally, more speculatively and in a style suited for a wider audience had they set out to compile a book. Aside from the cache of contributions on computation, they show primarily what can be achieved by technical expertise rather than creative imagination. Because John Archibald Wheeler has displayed, and continues to display, the power of the imagination more powerfully than almost any other physicist, that is a pity. But perhaps it shows simply that only John Wheeler can write the articles that John Wheeler writes. □

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On the beach

Donald J.P. Swift

The Morphodynamics of the Wadden Sea. By Jürgen Ehlers. A.A. Balkema: 1988. Pp.397. DM 185, £52.75.

THE Wadden Sea is the intertidal zone of the German Bight of the North Sea. Varying in width from 10 to 50 km, it is an expanse of tidal channels, flats, inlets, flood and ebb deltas, barrier islands and estuaries that extends from Den Helder in the Netherlands to Blåvandshuk in Denmark.

Jürgen Ehlers explains that while mapping the geomorphology of the Wangerooge (map) sheet, "I came to realize the importance of both the major and minor bedforms of the Wadden Sea.... At the same time, my observations led me to suspect that the remodeling of the landscape [was occurring] at a much faster rate than I had previously assumed". With his consciousness thus raised, Ehlers initiated an aerial photographic survey of the Wichter Ee, a tidal inlet between the barrier islands of Norderney and Baltrum, over a 30-day period. The resulting analysis of shoal and megaripple migration forms the core of this book, but there is much more.

After chapters on barrier-island development, recent geomorphological processes, morphodynamic units and historical development, the author rolls up his sleeves and launches into a description, one by one, of the 35 barrier islands of the Wadden Sea and their associated intertidal terrains. This roll call of islands, from Fanø, Mandø, Rømø and Sylt in the North Frisian Islands, through Wangerooge, Spiekerooge, Langeoog and Baltrum in the East Frisian chain, to Terschelling, Griend, Vlieland and Texel in the West Frisian Islands, will for many readers constitute the real strength of the book.

The careful review of historical records is another valuable contribution. Eight centuries of documents allow us to shift from the time scale of fluid dynamical processes at seconds, minutes, days and years into the lower portion of the geological frequency band. Within these eight centuries we can resolve some of the event-dominated fine structure of the most recent phase of the Holocene transgression of the North Sea basin. Important loss of land occurred in the storm surges of the years 1010, 1020, 1041, 1075, 1094, 1102, 1114, 1164 and 1282. The greatest single land loss resulted from the surge of 16 January, 1362, the second Marcellus flood, also known as the *Grosse Mandränke* (great mandrowning). Jade Bay, the Dollart and Harle Bay were enlarged, and in Nordfriesland vast areas of

land disappeared under water, including the legendary Rungholt, east of the present island of Pellworm. A second *Mandränke* occurred on 11 October, 1694. But the main and partially enduring land losses, resulting in the formation of Jade Bay, the Dollart and the Zuider Zee, did not occur as the result of single events, but gradually, through many smaller stages. These land losses were due to a lack of technical infrastructure capable of protecting the vast forelands from the destructive effects of later surges in later decades. Land reclamation occurred, but only through projects that lasted for centuries.

It is important to appreciate this monograph for the fine work of descriptive morphodynamics that it is, and to avoid

viewing it as an indifferently designed work of other purpose. The author's skills lie in the collecting and ordering of information. Chapters that attempt to take an overview, such as those on natural preconditions and barrier-island development, are not altogether successful, although they are always interesting. On the other hand, the relentless procession of maps, aerial photographs and, above all, photograph after photograph at ground level, has a hypnotic effect. Somewhere through the 393 figures, these vistas of misty dunes, beaches and marshes, and of tidal flats extending to the horizon, seep into the unconscious—you have *been* there. □

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Nature explained

R. McNeill Alexander

Life's Devices: The Physical World of Animals and Plants. By Steven Vogel. Princeton University Press: 1988. Pp.367. Hbk \$49.50; pbk \$17.95.

WHY do starfish have five arms? Why don't animals run on wheels? And isn't it intriguing that spider silk has five times the strength of mild steel, and that prairie dogs (rodents, not canines) dig self-ventilating burrows? Here is a brilliant and eccentric book that looks at living things from an engineering point of view, assuming astonishingly little previous knowledge of science on the reader's part.

A summary of one chapter will give the flavour of the whole. Under the same weight of laundry, a sagging clothes line is less likely to break than a tight one. This helps us to understand why there is more tension in the wall of a large pipe than of a smaller one, when both contain fluid at the same pressure: automobile tyres need thicker walls than bicycle tyres (although the latter are inflated to higher pressure), and arteries need thicker walls than blood capillaries. Toy balloons do not inflate uniformly but start with a localized bulge, and blood vessels would be apt to swell similarly were it not for the peculiar properties of their walls. Our lungs need an internal coating of a wetting agent to make their 30 million tiny pockets (alveoli) swell uniformly when we breathe in; which leads to an explanation of why bubbles form against the wall of a beer glass, not in mid-beer.

Professor Vogel suggests two uses for his book: as the basis of a course for non-scientists in liberal studies programmes or, for biologists, as a preparation for deeper biomechanical studies and more advanced reading. *Life's Devices* will be excellent in either role. It is organized by

physical topics: dimensions and scaling principles first, then fluid mechanics, properties of materials, structures and mechanisms. All the mechanics is introduced simply, including tricky topics such as the bending of beams. There are no logarithms or trigonometrical functions, and there is even an appendix as a reminder of how equations work. No background in science is necessary to enjoy the book, only intelligence and the willingness to stop and think.

Here are a great many fascinating scientific stories, including quite a lot that I did not previously know. Too often, though, the explanations are frustratingly short. I doubt whether naive readers will get a clear understanding of the plastron of aquatic insects, which enables them to breathe under water, from this brief, unillustrated account. I doubt whether they will appreciate the strange properties of slug slime, which enables slugs to crawl while keeping the whole area of the foot perpetually on the ground; as the slug crawls the slime under each part of its foot behaves alternately like a rubbery solid (giving the slug a purchase on the ground) and like a viscous liquid (letting it slide forward). The sparkle of the book depends on its pace, but there were times when it would better have been slowed down.

Professor Vogel is a distinguished biologist who has discovered many revealing things about the flow of water and air over animals and plants. He is also the author of a more advanced biomechanics book, *Life in Moving Fluids*, published by Willard Grant, Boston, in 1981. In an aside half way through his new book he tells us the secret of his success, which is also the book's philosophy: "A good way to make a neat discovery is to begin, not with your favorite organism, but with some physical property or phenomenon and then ask how animals might take advantage of it".

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A 140,000-year continental climate reconstruction from two European pollen records

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The dramatic and cyclic changes that have characterized the Earth's climate throughout the Quaternary may be illustrated by developments during the last such cycle which took place over the past 140,000 years. Quantitative reconstruction of the continental climate over this period improves the correlation between marine and land records and emphasizes the role of post-temperate forested episodes at the beginning of ice-sheet formation.

IN 1971, Imbrie and Kipp¹ used foraminiferal data obtained from deep-sea cores in palaeoclimate reconstruction. Since then, different multivariate techniques have been used to calculate palaeoclimate parameters from fossil biological data. For continental records, pollen analysis provides the most reliable data². Pollen is deposited as 'pollen rain' which can be characterized by a percentage representation of its components, the so-called 'pollen spectrum'. This provides a reliable reflection of regional vegetation³, and hence of regional climate. Past climates can therefore be established from the pollen content of sediments favourable to pollen preservation. Quantitative analysis of such records has until now provided palaeoclimate reconstructions for relatively short periods^{4,5}. Here we present the results of applying an improved and simplified method⁶, using pollen-stratigraphic records from La Grande Pile⁷ and Les Echets⁸, in eastern France, each containing a sediment sequence for the past 140,000 yr.

The results agree well with the palaeoclimate information derived from marine isotope data and emphasize the role of forested periods before glacial stadials as important episodes of ice-sheet formation. We also show that the arboreal pollen sum, often used by palaeoclimatologists as an index of continental temperature, is inadequate for this purpose.

Methods

We assume that different climates and their corresponding vegetation types and pollen spectra, which have succeeded each other through time in the study area, can be found today in similar, analogous forms. It follows, therefore, that in order for climates to be represented that are quite different from those at the studied locality today, a very extensive study area is required to provide a wide range of analogues. We therefore first establish representative modern pollen spectra from a large area with wide variations in present-day climate and vegetation; then we define precise climate data for all the sites from which pollen spectra are obtained and identify modern spectra that are most similar to each fossil spectrum. Finally we use modern climate parameters corresponding to the best analogues to infer past climatic conditions.

There are three main problems: The climate requirements of modern plants may be different from those in the past; the range of past climatic conditions may not be fully represented today; and past and present human activity has disturbed the vegeta-

tion/climate, and hence the pollen/climate relationships in the modern pollen data set.

It is unlikely, however, that any major evolutionary changes in trees or herbs (considered here at the genus level) have occurred over the past 140,000 yr. The second problem can be overcome to an extent by using the greatest variety of modern spectra available. We try to minimize anthropogenic effects by using a method for identifying the best analogue spectra; the percentages corresponding to each taxon have been attributed a 'loading factor' which expresses the role it has played at the site throughout the period covered by the reconstruction.

Data

The modern data, covering Europe, North Africa and Siberia, are represented by 227 spectra selected to represent a wide variety of vegetation types and climates. The 182 spectra described by Guiot⁶ are complemented by new data from Morocco, Tunisia, Italy and Corsica, so that a total of 52 taxa could be used.

Annual temperatures and precipitation were interpolated for localities from which the spectra were collected⁶. Among the taxa of fossil spectra, only the 52 taxa used in the modern spectra were considered.

The Grande Pile record (47°44' N, 6°30'14" E, 330 m above sea level) is from three cores⁷: 'Grande Pile 1' for the Holocene (depth: 55–320 cm), 'Grande Pile XIV' for the end of the last glacial (depth: 452–1,193 cm) and 'Grande Pile X' for older periods (depth: 1,243–1,865 cm). We analysed 268 pollen spectra and as "the miscellaneous and varying content of the pollen spectra from the section 1,170 cm–1,240 cm explains the hypothesis proposed by Gruger (1979) that they include quite a number of rebedded pollen, which deprives them of all botanical and hence climatological significance"⁸, we have neglected this particular section.

The Echets record (45°48'30" N, 4°55'20" E, 267 m above sea level) corresponding to the 39-m core of Les Echets G⁸, yielded 573 spectra (out of the 800 analysed). It was shown⁸ that the spectra from the episodes 'Melisey I' and 'Melisey II' were contaminated by reworked arboreal pollen (AP) from earlier temperate deposits, and so for these episodes the mesic AP frequencies were limited to mean values recorded at La Grande Pile for the same periods. As the Les Echets record does not include the Holocene, data for this period are derived from Hières-sur-Amby, 30 km to the south-east but at the same elevation⁹.

Results

First, we produced a time analysis of each spectra sequence to calculate how each taxon should be weighted as a climate signal. The modern ecological correlation between each taxon and climate cannot be used because of anthropogenic effects. When several well dated contemporaneous sequences are available, information that is common to all of them (mostly of a palaeobioclimatic nature) can be used⁶. In the present case, only the information conveyed from one level to another can be taken into account (Fig. 1a). However, such information is contaminated by noise from the local site peculiarities and (for about

the past 1,000 yr) human activity. Moreover the vegetation at any time is a function not only of the climate at that time but also of previous vegetation and climate.

Now, successive eigenvectors represent different parts of the autocorrelation (Fig. 1a), and therefore only the first eigenvector—in which climate is necessarily dominant—is retained; the others—in which noise is assumed to be dominant—are removed. Moreover, this first eigenvector maximizes autocorrelations and hence the persisting action of climate on vegetation.

The weight attributed to each taxon in the first eigenvector defines its particular role in the biological expression of the climate changes that occurred during the period concerned, and is termed the palaeobioclimatic operator (PBO). When applied to the fossil sequence, it provides a times series which can be considered as the best possible climate profile of vegetation changes.

There is a close correlation between the time series of La Grande Pile and Les Echets (Fig. 2), indicating that the operator, although derived from an autocorrelation analysis made at a single site, has a regional and therefore a climate value. This shows *a posteriori* the effective removal of any noise.

Figure 2 also shows the fluctuation of the AP sum often used by non-botanists as a palynological climate index. Note that the correlation between the AP sum at La Grande Pile and Les Echets, especially in relation to amplitude, is not as clear as that between the PBOs. In addition, comparison between the time series and the AP at each site (correlation coefficient $r = 0.87$ at La Grande Pile and 0.85 at Les Echets) shows two main differences: the PBO increases later and decreases earlier than the AP sum; and amplitude variations in the PBO are smaller

FIG. 1 The reconstruction method may be summarized as follows: a. The transfer of the climatic information from one level to the other is estimated by the first-order multiple autocorrelation matrix A_1 between the m taxa, computed on the n fossil spectra. The element (j, l) of A_1 is the cross-correlation coefficient between taxon j and l :

$$r_{jl} = \frac{1}{n} \left[\sum_{t=2}^n \frac{f_{jt} - \bar{f}_j}{Sf_j} \frac{f_{lt} - \bar{f}_l}{Sf_l} \right],$$

where f_{jt} represents the frequency (in percentages multiplied by 10) of taxon j in fossil spectrum t , the upper-bar represents the mean calculated across the fossil sequence and S is the standard deviation. As in principal component analysis—with a specific treatment to take into account its asymmetric character— A_1 is reduced to a few eigenvectors. The first eigenvector explains ~70% of the sum of squared autocorrelations (72% for La Grande Pile and 69% for Les Echets). The second eigenvector (and the succeeding ones), explaining <10% of this sum, are not retained. The weights w_j of each taxon in the first eigenvector (called PBO) are used in the second step, and also to provide the time series of the PBO (see Fig. 2): $b_t = \sum_{j=1}^m w_j (f_{jt} - \bar{f}_j) / Sf_j$, $t = 1, \dots, n$. b. A weighted euclidian distance operator, integrating the PBO, is used to measure the similarity between fossil and modern pollen spectra and to identify the best-fit analogues for the fossil spectra: $d_{it}^2 = \sum_{j=1}^m w_j^2 (\ln(f_{jt} + 1) - \ln(f_{ij} + 1))^2$, where t is the index of fossil spectra, i for modern spectra, and w_j is the PBO loading affected to taxon j . This set of k most similar modern spectra is denoted $(P_{t1}, \dots, P_{tk})$. However, because the modern data set may not include an exact analogue for each fossil spectrum, the single best-fit modern spectrum may not be appropriate. Instead, we prefer a Monte Carlo simulation of the data, whereby k spectra are randomly extracted with replacement from the k selected spectra, and the best-fit spectrum from that group is obtained. This is repeated s times, providing s analogues from which confidence intervals are calculated. c. The estimated past climate (analogue climate R_t) for a given analogue, indexed by k , from which the distance is d_{tk} is given by

$$^oR_t = \left(\sum_{k=1}^s C_k / d_{tk}^2 \right) / \left(\sum_{k=1}^s d_{tk}^{-2} \right).$$

The lower and upper limits of this mean estimate are

$$\begin{aligned} -R_t &= ^oR_t - \sqrt{(\sum_{k=1}^s C_k^2 / d_{tk}^2) / (\sum_{k=1}^s d_{tk}^{-2}) - ^oR_t^2} \\ +R_t &= ^oR_t + \sqrt{(\sum_{k=1}^s C_k^2 / d_{tk}^2) / (\sum_{k=1}^s d_{tk}^{-2}) - ^oR_t^2}. \end{aligned}$$

The probability covering the interval $[-R_t, +R_t]$ is defined by the proportion of modern analogous climate C_k accounted for within the range of s values.

than those in the AP during episodes of generally low forest cover.

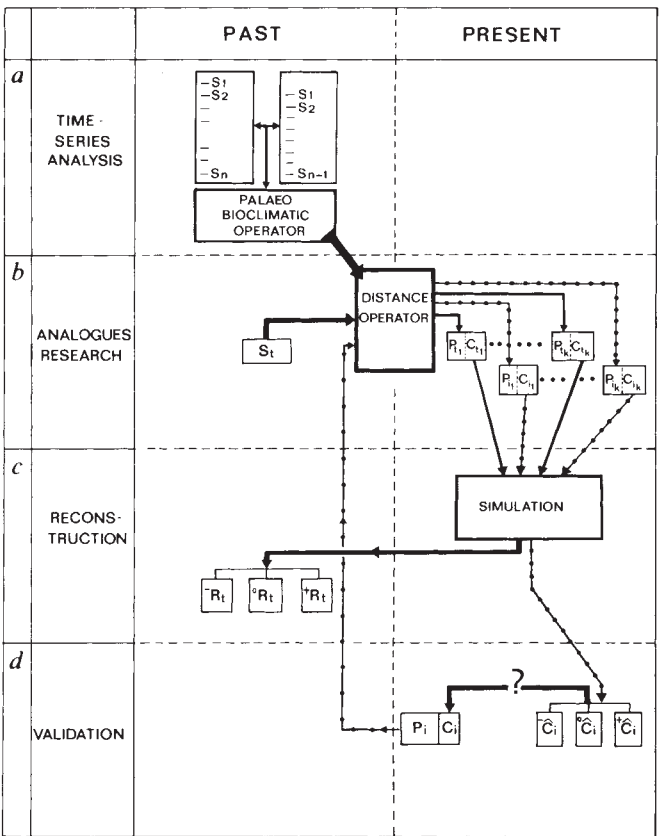
These differences arise because in the AP sum the pollen frequencies of all arboreal taxa are weighted equally whereas in the PBO the percentage of each tree taxon is weighted by its component weighting. For *Pinus* and *Betula*, this weighting may be very different from that of the other trees and close to the coefficient of non-arboreal taxa.

Figure 2 also shows that the variations in the PBO time series correspond more closely than do the AP curves to the oxygen isotope stages defined by Emiliani¹⁰ and Shackleton¹¹, represented by the stacked and smoothed record in the SPECMAP timescale¹².

We next look for analogues. A weighted euclidian distance ('distance operator'), in which the PBO provides a weighting for all pollen frequencies, is used to measure the similarity between ancient and modern spectra, thus enabling us to identify the best analogues for each fossil spectrum.

We then reconstruct palaeoclimate on the basis of the climatic characteristics of the best analogues. A Monte Carlo technique (Fig. 1b) enables us to derive a climate estimate with a confidence level (Fig. 1c) of about 70%. The confidence intervals average 180 mm and 2.1 °C (Table 1). These intervals are asymmetric and slightly larger for Les Echets.

Finally, we investigate both the validity of using a PBO and our method as a whole. We reconstruct climate parameters from modern pollen spectra for which values are known (Fig. 1d). The modern spectra are dealt with in the same manner as the fossil ones, and the reconstructed climatic parameters are compared to actual data (Table 1). The estimates correlate well (0.73 on average). The confidence intervals are slightly narrower than



This interval enables one to appreciate the quality of the analogues. This simulation procedure replaces the more complicated Kalman filtering previously used⁶. d. We use the entire procedure described in b and c, with index i replacing index t . The modern climate triplet $[C_i, ^oC_i, +C_i]$ estimated in this way is compared with actual climatic parameters C_i (see Table 1: modern data column).

TABLE 1 Validation statistics of the reconstructions

		Fossil reconstructions		Modern reconstructions	
		Precip. (mm)	T (°C)	Precip. (mm)	T (°C)
Grande Pile	+ME	173	2.1	138	1.8
	+ME	183	1.8	178	1.7
	Cor	—	—	0.77	0.70
Echets	+ME	156	2.2	141	1.6
	+ME	198	2.4	164	1.8
	Cor	—	—	0.75	0.71

Cor is the correlation between estimated and actual data, +ME is the mean upper standard deviation associated to the estimates ($+R-\sigma_R$ in Fig. 1c), -ME is the lower standard deviation ($-R-\sigma_R$ in Fig. 1c). These statistics are calculated on the fossil data and on the modern data. In this last case R must be replaced by C (see Fig. 1d).

those computed on fossil data (155 mm and 1.7 °C on average) and have a higher confidence level (>90%). Such good agreement is particularly valuable as the modern estimates are based upon coefficients calculated solely from the fossil data.

The confidence interval mean is larger than that of other reconstructions (13, for example) based upon assumptions of a gaussian distribution and homogeneity between modern and fossil data. As these assumptions are never exactly satisfied, a more appropriate method, including a simulation approach, is used. Such a method provides less optimistic but more realistic confidence intervals. Note, however, that a part of the confidence interval reflects the variability of the calibration data set, so that the cold and dry periods have larger confidence intervals than the warm and/or humid ones. This suggests that the major weakness in our reference data set is a relative lack of cold and dry analogues, which of course it is difficult to compensate for, during the present temperate period.

Discussion

The reconstructions are obtained as a function of depth. To facilitate comparison with reconstructions from deep-sea cores,

the results are presented on a timescale (Fig. 3). The most important events before 30,000 yr BP are dated on the basis of the widely accepted isotopic chronology¹², whereas ¹⁴C ages^{8,14} are used to date events after 30,000 yr BP (Fig. 2). Intermediate dates are linearly interpolated with steps of 1,000 yr.

We are aware that this procedure does not ensure the independence of our chronology with respect to that of the isotopic stratigraphy. But the succession—if not the chronology—of the climate phases shown by the pollen record is independent, and so are the reconstructed values even though the variation in time is, of course, derived from isotopic records.

The temperature and precipitation curves obtained at both sites agree well. The Holocene and the last interglacial (Eemian) appear clearly as the two warm and humid episodes which followed a complex period marked by significant rises in temperature and precipitation.

A temperature drop, which can be seen during the latter half of the Eemian and is clearly reflected in the vegetation changes, precedes a moisture maximum.

In agreement with botanical evidence, we find that a clear succession of two relatively warm and humid periods occurred after the Eemian. The first, the St-Germain I interstadial, appears as a climatically complex period because at both sites it is interrupted by relatively cold and humid short episodes and the latter half of this period is characterized by a precipitation maximum following a marked fall in temperature.

We found the two periods following the Eemian and the St-Germain I interstadial (the Melisey I and the Melisey II stadials, respectively) to be cold and dry, as expected.

For the second temperate period (the St-Germain II interstadial), we find warm and humid conditions overall, but with a tendency for low temperatures and high precipitation towards the end of the period.

The following period, the Lower Würmian Pleniglacial, was initially humid and cold, then very cold and very dry, as were the 'late Eemian-Melisey I' and the 'late St-Germain I-Melisey II' periods.

The Middle Würm then followed, a dry and cold period but far less severe than the previous stadials. At Les Echets, two

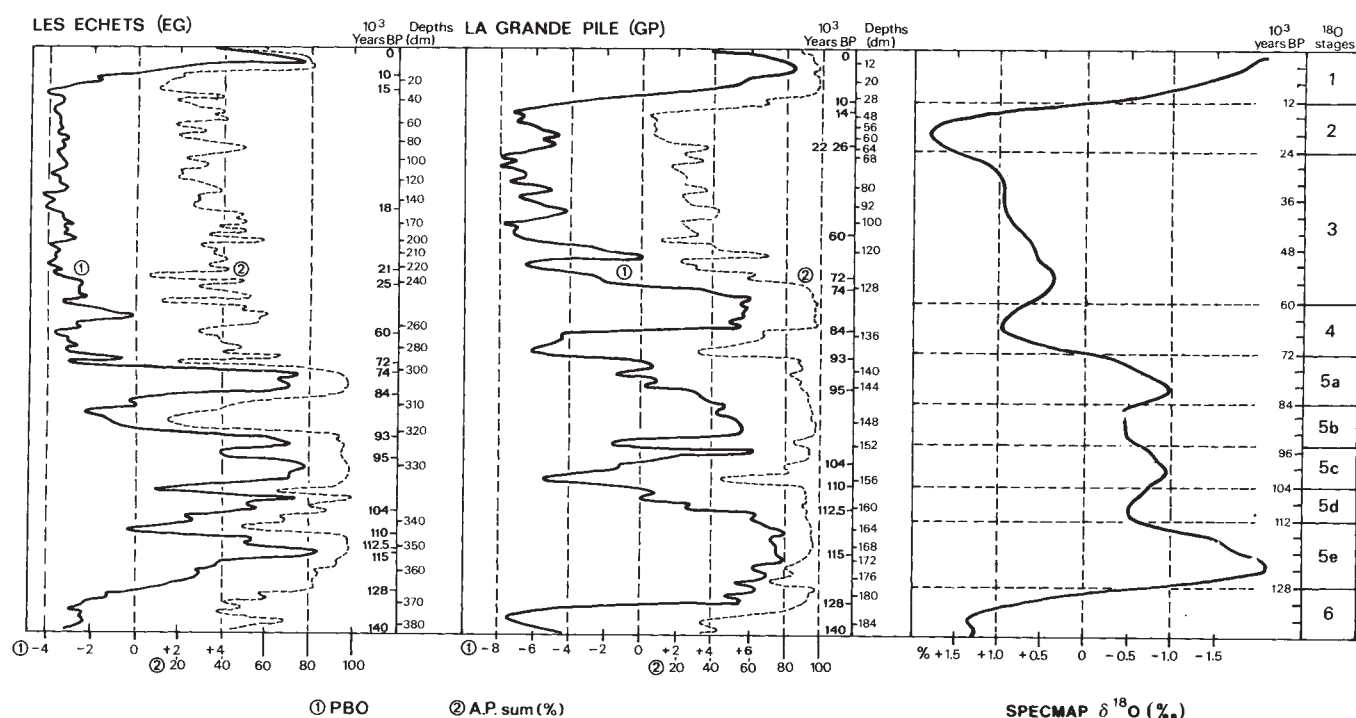


FIG. 2 The palaeoclimatic operator time series, and the arboreal pollen (AP) sum (%) for Les Echets and La Grande Pile. These series are compared with the stacked, smoothed oxygen isotope record as a function of age using

the SPECMAP scale¹². The vertical axis is linearly related to the spectra numbers. Estimated ages are given with regard to the depths.

short temperate episodes comprised a rather cool middle phase, and at La Grande Pile we found the same division into two relatively temperate episodes with an intermediate cooler one. These results support the case for a threefold division of the Middle Würm, as suggested by European Atlantic micro-palaeontological data¹⁵ and insolation curves¹⁶. These results, however, need to be verified because they suggest climate parameters which, throughout this period (from ~60,000 to ~30,000 yr BP), are either fluctuating (Les Echets) or poorly marked (La Grande Pile).

Because of differences in the sediments, it is difficult to compare results for the two sites for the Upper Pleniglacial. Climate, during this period, was chiefly characterized by continuous low temperatures, no such equivalent being found since the beginning of the Eemian.

Low-amplitude oscillations, mainly in relation to moisture, are recorded repeatedly, especially at Les Echets, but on the strength of botanical and sedimentological data, they are believed to result from local fluctuations rather than regional general climate variations¹⁷.

The Younger Dryas is not present in the available record of La Grande Pile and is poorly marked in the Les Echets/Hière-sur-Amby succession.

Implications

If one agrees with Ruddiman and McIntyre¹⁸ that an accumulation of continental ice implies a cold and humid continent, as opposed to a hot Atlantic Ocean between latitudes 50° and

60° N, the results presented here suggest that there were three major ice-accretion periods in Europe.

The first corresponds to the very humid and markedly cold climate of the final part of the Eemian, a prelude to the even colder and dry Melisey I stadial. The second is the end of the St-Germain I interstadial (very humid but moderately cold) which was succeeded by the cold and dry Melisey II stadial. The third ice-accretion period corresponds to the end of St-Germain II interstadial and to the beginning (markedly cold but moderately humid) of the Lower Pleniglacial, before the second very cold, dry part of this major stadial.

The main ice-accretion period, which brought about an 'inception' period¹¹ (increase of global ice above modern values), started before 110,000 yr BP, the date cited as the end of the Eemian Interglacial¹². Note that the date 115,000 yr BP corresponds to minimal insolation in June, July and August at 60° N (ref. 16). A comparison of the Earth's orbital parameters at 115,000 yr BP with those of 125,000 yr BP suggests a cooling of the soil and an increase of soil moisture¹⁹ in regions situated between the Mediterranean Sea and Siberia.

Isotopic and pollen analyses of an eastern Atlantic core have shown that the fall in the oxygen isotope curve based on benthic foraminifera—the beginning of the first ice-growth phase somewhere within polar latitudes—was contemporaneous with the expansion of *Carpinus* in Europe²⁰. However, our results suggest that in Europe, the first glacial accretion began only after the *Abies* forestation episode, which followed the *Carpinus* forestation episode. This accretion, evident in foraminifera $\delta^{18}\text{O}$

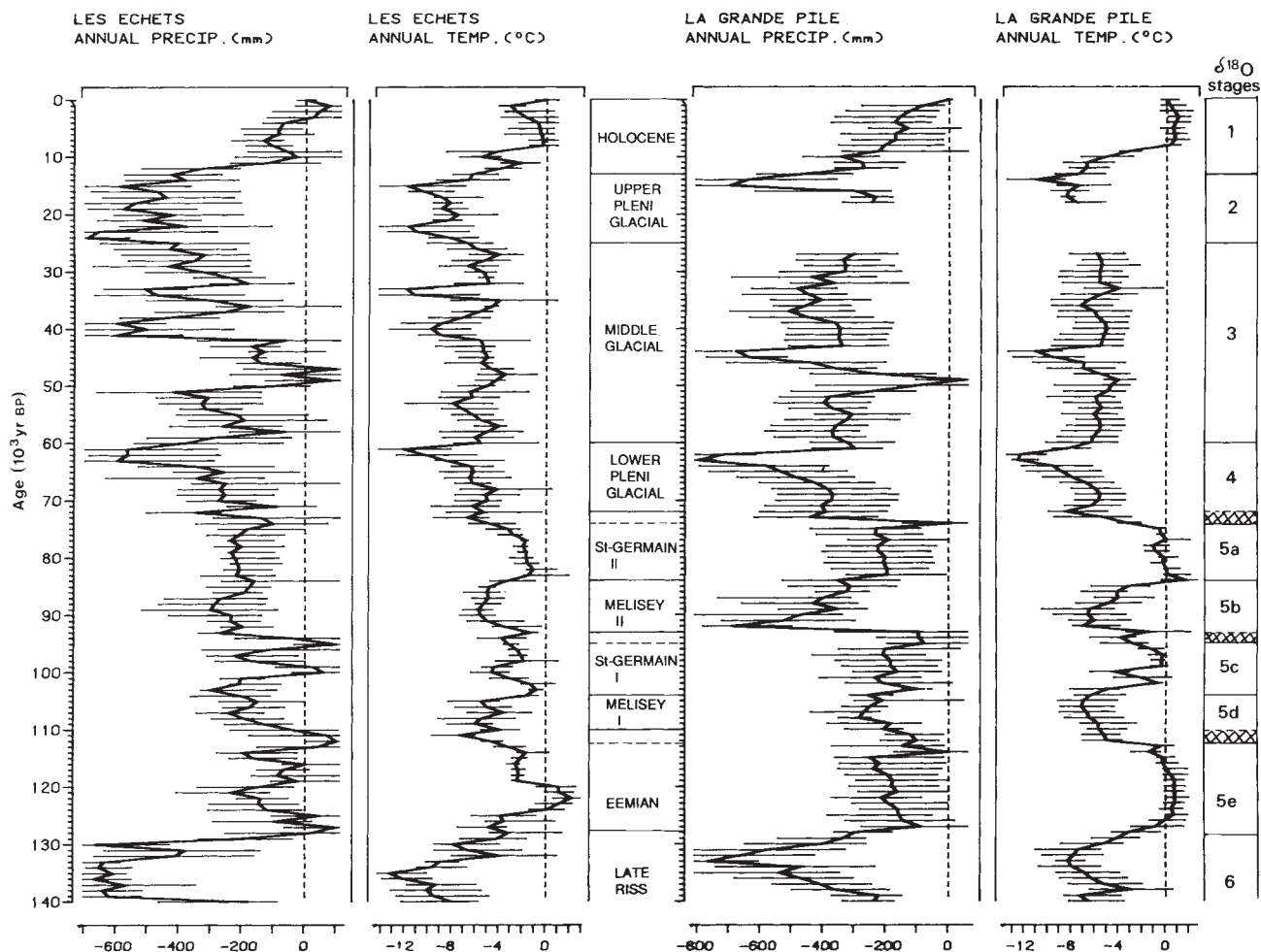


FIG. 3 Reconstruction of variations in annual total precipitation and mean temperature, expressed as deviations from the modern values (1,080 mm and 9.5 °C for La Grande Pile, 800 mm and 11 °C for Les Echets). The error

bars are computed by simulation. The vertical axis is obtained by linear interpolation from the dates indicated in Fig. 2.

data, should therefore be located in the north-west Atlantic. Changes in insolation that occurred around 115,000 yr BP and which are considered to account for this event²⁰ would then have brought about the glacial accretion on the European side of the Atlantic, with a substantial delay, as has been suggested previously²⁸. As our chronology depends on the isotopic chronology, however, we cannot say how long this delay was.

It is generally advocated (for example, refs 14 and 20) that marine isotopic stages or sub-stages characterized by low $\delta^{18}\text{O}$ values correspond exactly with stadial or pleniglacial continental episodes. Our reconstruction shows, during the first part of the last climatic cycle, well characterized transitions between periods of either low oceanic $\delta^{18}\text{O}$ values (interglacial or interstadial climatic optimums) or high ones (stadials or pleniglacial). As a result, the lower limit of the continental equivalent of isotopic sub-stages 5d and 5b and of stage 4 should be situated within transition periods and not correlated exclusively and directly with the Melisey I and Melisey II stadials or the Lower Pleniglacial, so that our results are more consistent with the interpretation of the marine isotopic stratigraphy concerning the duration of substages 5d and 5b and of the stage 4 than is the previous interpretation of the continental climatic record^{14,20}. It is

noteworthy that the transition periods, which play a major part in initiating ice-sheet formation, are forested periods. They correspond to *Picea*, *Pinus* and *Betula* forests, which mark the latter part of temperate episodes that follow the warm climate optimum; such forestation episodes are therefore often called 'post-temperate'.

The palaeobioclimatic operator appears to be the best botanical indicator of climate change, and should be preferred to the AP sum, which is unreliable, particularly for estimating palaeotemperatures. However, our reconstruction clearly suggests quite temperate conditions for the St-Germain I and St-Germain II interglacials, conditions almost similar to those of the present day, especially in relation to temperature. Such temperate phases are not recorded in the Antarctic ice cores²², in Pacific Ocean records²³, or in Atlantic Ocean deep-water temperature estimates²¹. More surprisingly, neither are they found in records from northern Europe^{24–26}. This might indicate a steeper thermal gradient for these periods than for today. By applying our method to pollen sequences from Padul (Andalusia)²⁷ on the one hand and to successions which contain records of these interstadials in northern Europe on the other, it should be possible to test this hypothesis. □

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The *Caenorhabditis elegans* heterochronic gene *lin-14* encodes a nuclear protein that forms a temporal developmental switch

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During wild-type development, a protein product of the *Caenorhabditis elegans* heterochronic gene *lin-14* is localized to nuclei of specific somatic cells in embryos and early larvae, but is absent in late larvae and adult soma. Gain-of-function *lin-14* mutations cause the level of *lin-14* protein to remain high throughout development, resulting in developmental reiterations of early cell lineages. The normal down-regulation of the *lin-14* nuclear protein level encodes a temporal switch between early and late cell fates.

DURING the development of multicellular organisms, genes specify the proper developmental fates of cells in particular spatial domains^{1,2} or particular cell lineages^{3,4}; mutations in such genes transform the fates of cells into those normally found at different positions or lineages. In *C. elegans*, the temporal pattern of the cell types generated during development is explicitly controlled by heterochronic genes in an analogous manner^{5–7}. Heterochronic mutations cause particular cells in various cell lineages and tissues to adopt fates during post-embryonic development that are normally associated with cells at earlier or later stages of development. An analysis of mutations in the heterochronic gene *lin-14* has indicated that this gene plays a central role in controlling the temporal pattern of the *C. elegans* post-embryonic cell lineage^{6,7}. Loss-of-function *lin-14* alleles cause the precocious appearance, during early larval

stages, of cell lineages that would normally be observed in descendent cells one or two larval stages later. Conversely, gain-of-function *lin-14* alleles affect the same cell lineages but cause the opposite transformations in cell fate; thus, the early cell lineages are normal, but later cells reiterate the early cell lineages normally associated with their ancestor cells. These results indicated that relatively high *lin-14* gene activity early in development is normally reduced later in development, and that this change in *lin-14* gene activity controls a switch from early to late cell fates in all of the cells affected by *lin-14* mutations⁷.

The *lin-14* gene has now been cloned using a restriction fragment length polymorphism (RFLP) mapping approach⁸. Here we use antibodies to the *lin-14* protein to observe the cellular and subcellular location of the *lin-14* protein and the temporal regulation of its accumulation during wild-type and *lin-14* mutant development. We find that the *lin-14* protein is localized in the nucleus and that its progressive elimination during *C. elegans* development controls the normal temporal sequence of cell fates mediated by the *lin-14* gene.

Lin-14 protein is nuclear and temporally regulated

To overproduce the *lin-14* gene product in *Escherichia coli*, we fused the *lacZ* gene of plasmid pUR290 (ref. 9) in phase with an open reading frame from a *lin-14* cDNA clone, number 557. The *lacZ-lin-14* fusion protein was partially purified by preparative SDS gel electrophoresis and used to immunize rabbits. The resulting antisera were affinity-purified using a *lacZ-lin-14*

fusion protein column. Figure 1a shows that this antibody preparation is *lin-14*-specific.

To detect the *lin-14* protein in *C. elegans*, cell extracts were prepared from staged cultures of *C. elegans* and analysed by immunoblotting using the anti-*lin-14* antibodies. A protein of relative molecular mass (M_r) 70,000 (70K) was detected that is most abundant in early larval stage animals but not in later stages (Fig. 1b). This 70K protein is absent in two *lin-14* null mutants (Fig. 1c), showing that it corresponds to (or, less probably, is positively regulated by) the *lin-14* gene product.

We used the affinity-purified anti-*lin-14* antibodies to detect the *lin-14* protein in whole-mount fixed specimens of wild-type *C. elegans* by indirect immunofluorescence staining, and found that the anti-*lin-14* antibodies bound antigen in specific somatic nuclei of late embryos and early larvae (Fig. 2). No immunofluorescence in somatic cells was detected in strains bearing the loss-of-function *lin-14* alleles *n536n540* and *n355n726*, which by genetic criteria behave as null alleles⁷ (data not shown), showing that in wild-type specimens the anti-*lin-14* antibody binds the *lin-14* protein itself (or, less probably, a gene product positively regulated by *lin-14*). Because both the 70K protein detected on the immunoblots and the somatic nuclear *lin-14* staining disappear in these *lin-14* mutant strains, the somatic nuclear staining probably corresponds to this 70K *lin-14* protein.

Mutations in *lin-14* change the developmental fates of certain post-embryonic blast cells^{6,7} (Fig. 3). By determining precisely which cells stain with the anti-*lin-14* antibody, we correlated

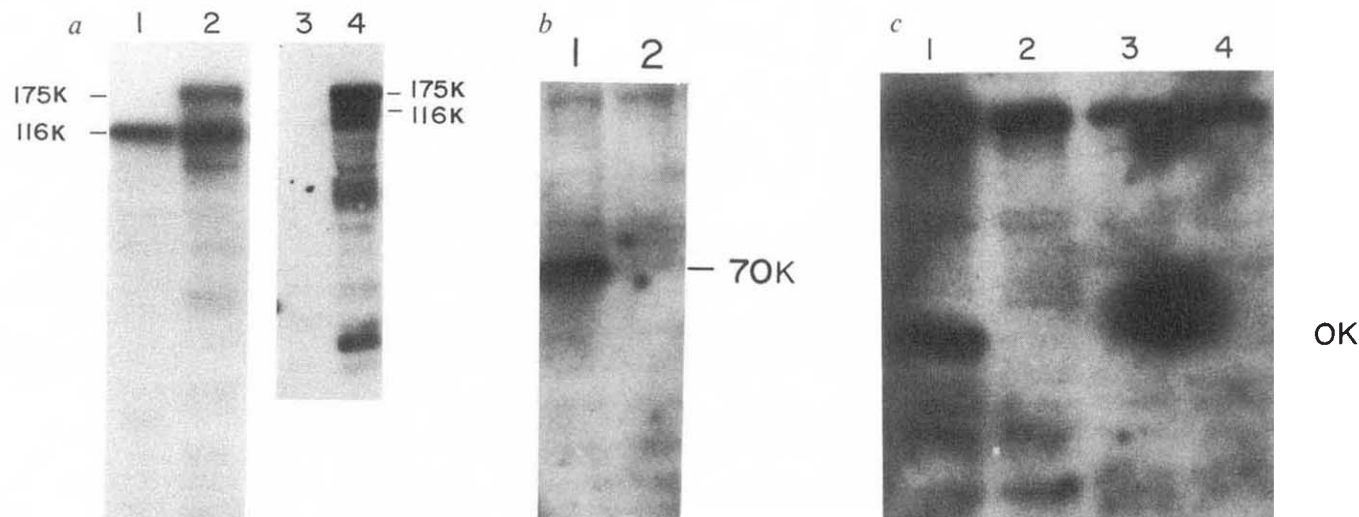


FIG. 1. *a*, Specificity of antibodies to *lin-14* on immunoblots. Lanes 1 and 3, protein extracts of IPTG-induced JM101/pUR290 cells. Lanes 2 and 4, protein extracts of IPTG-induced JM101/pUR290-cDNA557 cells. Lanes 1 and 2 were probed with sera partially purified by affinity chromatography with protein extracts from *E. coli* expressing the *lacZ-lin-14* fusion gene. The partially-purified sera recognizes both *lacZ* (lane 1) and *lacZ-lin-14* (lane 2) epitopes. Lanes 3 and 4 were probed with further purified sera from which anti-*lacZ* and anti-*E. coli* antibodies had been removed by affinity chromatography with protein extract from *E. coli* expressing *lacZ*. This sera detects only the *lacZ-lin-14* fusion protein (lane 4) (Lower M_r bands are presumably degradation products of this fusion protein that retain *lin-14* epitopes.) This sera was used for all immunological detections of *lin-14* protein in *C. elegans* protein samples and fixed specimens. The M_r of the *lacZ-lin-14* fusion protein is 175 K. *b*, Detection of the *lin-14* protein in extracts of wild-type *C. elegans*. The sera specific for *lin-14* was used to probe a western blot of protein samples isolated from staged *C. elegans* strains: lane 1, wild-type (N2) larval stage 1; lane 2, wild type (N2) larval stage 2. *c*, Lack of *lin-14* protein in two *lin-14* null mutants: lane 1, wild-type (N2) larval stage 1; lane 2, *lin-14(n536n540)* larval stage 1; lane 3, *lin-14(n355n726)* larval stage 1; lane 4, *lin-14(n355n726)* larval stage 3. The large protein on this blot was detected in both wild-type and *lin-14* mutants with some antibody preparations. Because it is not affected in these *lin-14* mutants, it may correspond to a cross-reacting protein.

METHODS. A cDNA library from *C. elegans* mixed-stage mRNA was constructed in λ gt10 using standard protocols¹⁷ and screened with *lin-14* genomic clone probes. The *lacZ-lin-14* fusion gene was constructed by ligating *Sal*-digested pUR290⁹ DNA to *Xho*I-digested *lin-14* cDNA557 DNA using standard techniques. The antibody was generated by immunizing New Zealand White rabbits with 500 μ g of *lacZ-lin-14* fusion protein per rabbit at 20 sites. To affinity-purify the sera, total protein extracts from JM101/pUR290 *Sal*-*Xho*I-cDNA557 and from JM101/pUR290 were each bound to derivatized beads (Affigel-10, BioRad)¹⁸. The sera was first adsorbed to the *lacZ-lin-14* affinity column, and subsequently, anti-*lacZ* and anti-*E. coli* protein antibodies were removed by exhaustive adsorption of this sera with the JM101/pUR290 protein extract affinity column. Western blots to *E. coli* extracts containing *lacZ* or *lacZ-lin-14* protein¹⁹ used a 500-fold or 1,000-fold dilution of the affinity purified anti-*lin-14* antibody. Antibodies were diluted 50-fold or 100-fold for western blots to *C. elegans* extracts. Bound antibodies were detected using ¹²⁵I-*Staphylococcus aureus* protein A (New England Nuclear) at 5 μ Ci ml⁻¹ (ref. 19).

the temporal and cellular pattern of *lin-14* protein accumulation with the cell lineages affected by mutations in the *lin-14* gene and with the known developmental fates of all those cells.

The *lin-14* protein was first observed in embryos at about 7 hours after fertilization or about half-way through embryogenesis, after which time most of the embryonic cells had been generated, and cell differentiation and morphogenesis were taking place¹⁰. At this stage, the most intense staining was in intestinal and hypodermal nuclei (Fig. 2a). In later stage embryos, at about 9 hours after fertilization, additional weak neuronal staining was observed (data not shown). No lineage defects before larval stage one (L1) have been observed in *lin-14* mutant strains, and the temperature-sensitive period for *lin-14* lineage defects occurs after hatching⁷, indicating that the accumulation of the *lin-14* protein detected in these embryos is not yet functional.

The most intense staining that we observed at any stage was in nuclei of late embryos just before hatching, and in newly hatched L1 animals. Significantly, only nuclear staining was observed at these stages (Fig. 2b, c). In the newly hatched L1 animal, the *lin-14* protein was present in the nuclei of most of the post-embryonic blast cells; intense nuclear staining was observed in the hypodermal blast cells H1, H2, V1-V6, and T (Figs 2c and 3) and in all of the intestinal (E) cells (Fig. 2b), and weaker staining was observed in both neuroblasts Q1 and Q2, in the mesoblast M cell, and in the P cells. No staining was observed in the somatic or germ line gonadal blast cell nuclei (Z1 to Z4) at this stage.

During the L1 stage, the hypodermal blast cells H1, H2, V1-V6, T, and intestinal cells, E, all divide¹¹. The nuclei of these progeny cells also stain with the anti-*lin-14* antibody during the mid-L1 stage. At this stage the P-cell nuclei migrate into the ventral cord and the cells divide¹¹. The *lin-14* staining in the P-cell nuclei fades before their migration into the ventral cord but reappears later in some of their progeny cells (see below).

The intestinal (E), hypodermal (H1, H2, V1-V6, T), neuroblast Q1 and Q2, mesoblast (M), and P-cell lineages are all affected by *lin-14* mutations; loss-of-function *lin-14* mutations cause these cells to precociously express late stage patterns of cell lineages⁶. Wild-type blast cells accumulate the *lin-14* protein before the first cell lineage defects can be observed during the L1 stage in *lin-14* loss-of-function mutants and before the L1 temperature-sensitive period of some *lin-14* mutations⁷. The gonadal blast-cell lineages are not affected by *lin-14* mutations⁶ and do not accumulate the *lin-14* protein. Thus, all post-embryonic blast cells affected by *lin-14* mutations also accumulate the *lin-14* protein at the expected time, and those post-embryonic blast cells not affected by *lin-14* mutations do not accumulate the *lin-14* protein.

The nuclei of many cells that do not divide post-embryonically also accumulate the *lin-14* protein during the L1 stage. The embryo-derived nuclei in the hypodermal syncytial cell hyp7, ABapppppa, ABplapppp, Cpaaaa, Cpaapa, Cpaapp, Cpapaa, all accumulate levels of the *lin-14* protein similar to those of the hypodermal blast cells (Figs 2c and 3). Terminally differentiated nuclei from embryonic body muscle also accumulate the *lin-14* protein. Nuclei of many but not all neuronal cells stain with the anti-*lin-14* antibodies. For example, the neurons BDU, ALM and CAN accumulate the *lin-14* protein but the HSN neuron does not (Fig. 2c, data not shown). All of the embryonically generated ventral-cord neurons, and some but not all of the neurons of the nerve ring and posterior ganglion accumulate the *lin-14* protein in their nuclei during the L1 stage (Fig. 2c, d, e).

These embryonic hypodermal, muscle, and neuronal cells are not affected by *lin-14* mutations but nevertheless accumulate the *lin-14* protein⁶. In the characterization of the *lin-14* mutants, changes in the patterns of cell division and differentiation of only blast cells, which divide post-embryonically, were scored; the effect of *lin-14* mutations on non-dividing cells was not

assessed. It is not known, therefore, whether the *lin-14* expression that we observe in these differentiated cells is in fact functional.

Late in the L1 stage, the *lin-14* protein staining of all nuclei except the neuronal nuclei is much weaker. At this stage more neurons of the nerve ring and posterior ganglion stain than at earlier stages (data not shown). Thus, in the hypodermal and intestinal cell lineages, *lin-14* protein levels peak during early L1 and fade entirely by L2. In the many neuronal cells, *lin-14* protein levels peak during mid to late L1 and fade by L2. By L2 and in subsequent larval stages, most animals showed no *lin-14* staining at all (Fig. 2f, g). The disappearance of the *lin-14* protein during the L1 stage from the nuclei of the post-embryonic blast cells correlates well with the mid to late L1 temperature-sensitive period for *lin-14* mutations⁷.

The temporal regulation of *lin-14* protein accumulation is displaced from this L1 peak in some cell lineages. The Pn.p cells, which do not divide until the L3 stage, do not accumulate the *lin-14* protein until that stage when they show very weak staining before the division (data not shown). This staining fades by early L4. The accumulation of the *lin-14* protein in the Pn.p cells during L3 correlates with the precocious Pn.p cell divisions, abnormal Pn.p-derived cell lineages, and resulting abnormal morphogenesis of vulval structures in *lin-14* mutants⁶. The temperature-sensitive period for this defect has not been determined.

In occasional L2 and L3 stage animals, weak *lin-14* protein staining in hypodermal, neuronal, and intestinal cells was observed in nuclei and cytoplasm, as if it was being slowly degraded or not efficiently transported to the nucleus. Patches of *lin-14* staining in hypodermal or intestinal nuclei was only rarely observed in very old adults (data not shown). Such sporadic accumulation of the *lin-14* protein indicates that the gene may be re-expressed under some unknown physiological conditions.

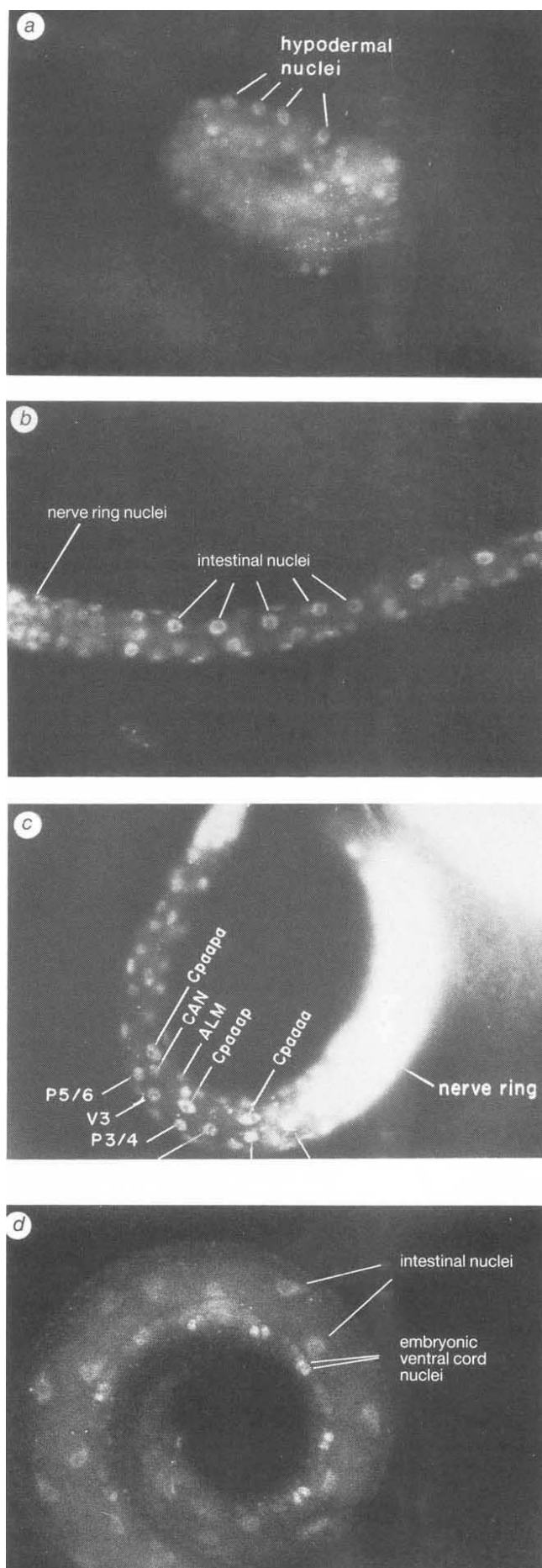
In most adults, *lin-14* immunostaining reappears only in the mature oocyte nuclei of hermaphrodites (Fig. 2h), at meiotic prophase I when the chromosomes are condensed as shown by DAPI staining (Fig. 2i). This oocyte expression of *lin-14* was not predicted by genetic analysis; no *lin-14* maternal effect has been detected (V. Ambros, personal communication). The oocyte nuclear immunofluorescence, however, remained in the *lin-14* loss-of-function mutant strains *n536n540* and *n355n726* (data not shown), indicating that these are not true null mutations for all tissues that express *lin-14*, or that the oocyte-specific antigen detected is not in fact a product of the *lin-14* gene, but rather an antigenically related gene product. The oocyte *lin-14* nuclear staining disappears after fertilization; presumably it is either degraded or greatly diluted during nuclear division. No staining is visible in early embryos.

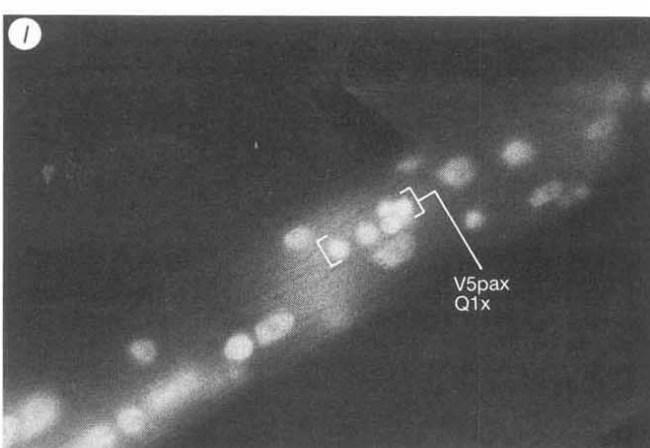
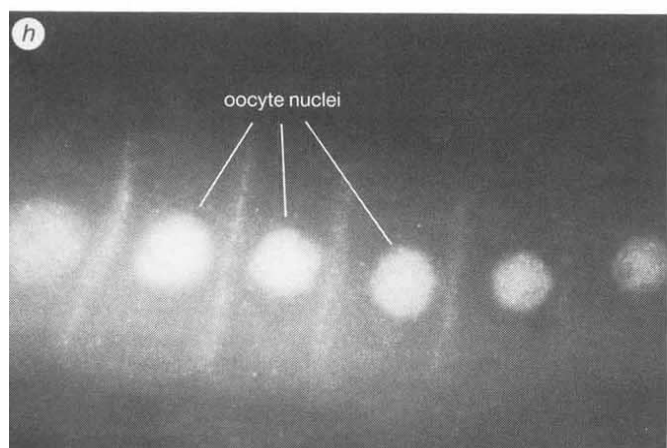
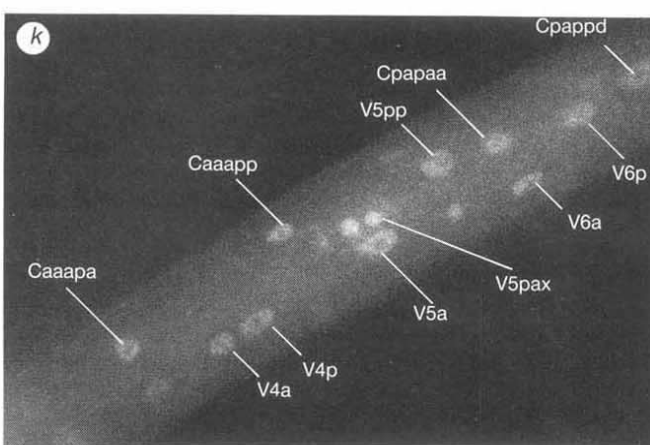
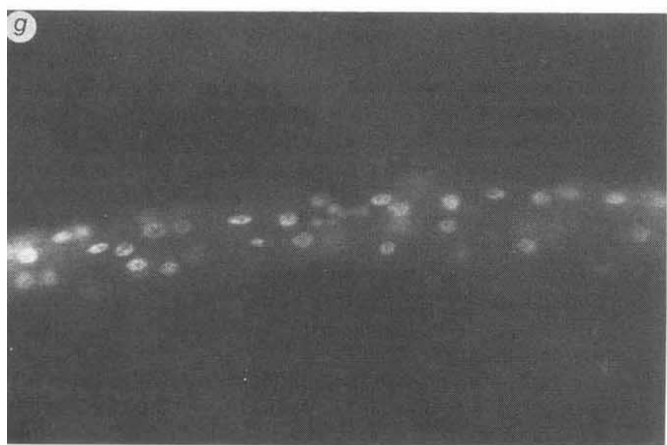
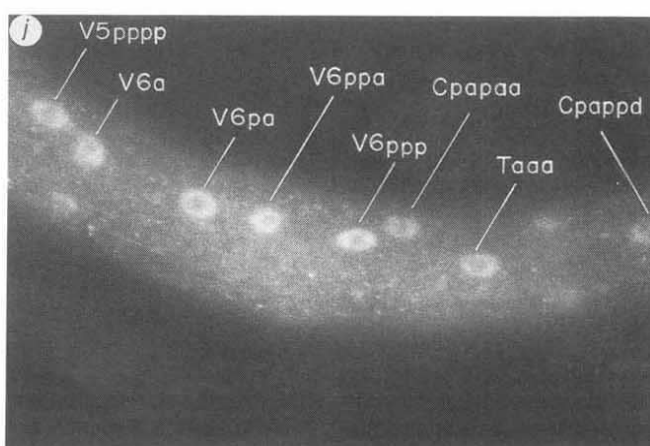
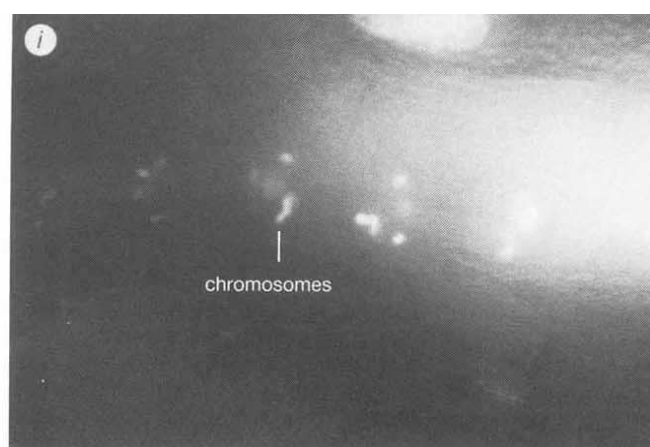
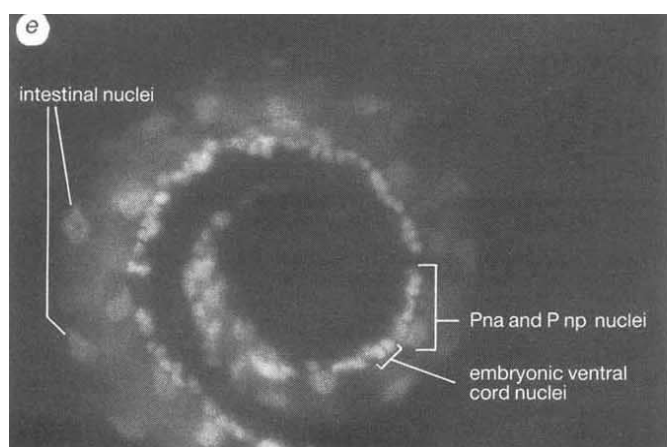
***Lin-14* mutations**

Gain-of-function *lin-14* mutations cause reiteration of early larval cell lineages at late larval and adult stages^{6,7}. The embryonic and L1 stage staining in these mutants was equivalent to that seen in the wild-type in its intensity, nuclear localization, and cellular distribution (data not shown). Unlike the wild-type, however, these mutants showed high levels of the *lin-14* protein in many nuclei during larval stages 2, 3 and 4 and in adults (Fig. 2j, k, l). Protein staining was also observed in hypodermal, intestinal, muscle and neuronal nuclei in these late larvae and adults. All of the post-embryonic blast cells known to be affected by gain-of-function *lin-14* mutations inappropriately accumulate the *lin-14* protein at these late stages. For example, during the L3 stage, the hypodermal nuclei V4.ppp, V5.pppp and Tapapap, which in these mutants will divide like the wild-type cells V4, V5 and T, respectively⁶, accumulate the *lin-14* protein (Fig. 2j). The abnormal presence of the *lin-14* protein in these cells seems to change their fates into those of V4, V5 and T, respectively. Similarly, in the adult stage, the cell V4.pppp divides inappropriately in these mutants⁶ and also accumulates

FIG. 2 Immunodetection *in situ* of the *lin-14* protein. Specimens were fixed, made permeable and incubated with affinity-purified rabbit anti-*lin-14* antibody followed by secondary fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit antibody and 4,6-diamidino-2-phenylindole (DAPI)-stained to visualize all nuclei. Immunofluorescent photographs taken with FITC filters to show *lin-14* protein staining or DAPI filters to show all nuclei are shown. All views are lateral. *a*, FITC, wild-type embryo at about 7 hours of embryogenesis. Bright nuclear *lin-14* protein staining can be seen in hypodermal nuclei. *b*, FITC, wild-type larval stage one (L1) animal with the plane of focus at the midline, showing intense *lin-14* protein staining in the large intestinal nuclei. Note that there is no nucleolar *lin-14* protein staining. Below the intestinal nuclei, the smaller nuclei of the ventral cord neurons stain with the antibody. Towards the anterior the *lin-14* protein staining nuclei of the nerve ring can be seen. *c*, FITC, wild-type early L1 animal with the plane of focus at the lateral hypoderm, showing protein staining in the lateral hypodermal blast cells V1, V2, and V3, the ventral blast cells, P1/2, P3/4, and P5/6, the neurons CAN and ALM, the embryonic hypodermal nuclei Cpaapa, Cpaap and Cpaaaa, and in the neuronal nuclei of the nerve ring. *d*, FITC, wild-type mid-larval stage-one animal with the plane of focus at the midline, posterior towards the inside of the spiral. On the inner edge of the spiral, pairs of *lin-14* protein staining nuclei from ventral cord neurons can be seen. Interspersed between these *lin-14* staining nuclei are the Pn.a-derived neural nuclei and Pn.p ventral hypodermal nuclei that do not accumulate the *lin-14* protein at this stage. The larger *lin-14* protein-staining nuclei are intestinal nuclei before the L1 division. *e*, DAPI, same animal as *d*. Note that derived in *d* many Pn.a ventral-cord neuron nuclei have not yet expressed the *lin-14* protein. *f*, FITC, Wild-type larval stage two (L2) animal with the plane of focus at lateral hypoderm. No *lin-14* staining is visible. *g*, DAPI, same animal as in *f*. View showing nuclei of the hypoderm and postdeirid neurons. A comparison of *f*, *g* with *j*, *k* shows the difference in a *lin-14* gain-of-function mutant. *h*, FITC, wild-type adult animal gonad. Shown are six mature oocyte nuclei that stain with the anti-*lin-14* antibody. Note also the lack of *lin-14* protein-staining in nuclei of somatic cells. *i*, DAPI, same animal as in *h*. The same six oocyte nuclei stained with DAPI show condensed chromosomes. *j*, FITC, Gain-of-function mutant *lin-14(n355)* larval stage three (L3) animal with the plane of focus at lateral hypoderm. Shown are hypodermal nuclei that contain the *lin-14* protein. The hypodermal nuclei contain a non-staining nucleolus. *k*, FITC, *lin-14(n536)* gain-of-function mutant larval stage three (L3) animal with the plane of focus at lateral hypoderm. *lin-14* protein staining can be seen in hypodermal nuclei (large) and neuronal nuclei (small) of the postdeirid, V5.pax. *l*, DAPI, same animal as in *k*. Note that in *k* only two of the five neuronal nuclei shown in this animal have accumulated *lin-14* protein.

METHODS. Indirect immunofluorescence on fixed *C. elegans* specimens: strains N2, MT355=(*n355*); MT1149=(*n536*); MT1153=(*n536n540*); GR200=(*n355n726*). About 0.1–0.5 ml of these animals were fixed in 5 ml of 2% paraformaldehyde, 80 mM KCl, 20 mM NaCl, 2 mM EGTA, 0.5 mM spermidine HCl, 0.2 mM spermine, 0.5% β -mercaptoethanol, 15 mM PIPES pH 7.4, to which 3 ml of *n*-heptane and 1 ml of 90% methanol, 10% 0.25 M EGTA was added²⁰. This emulsion was shaken for 20–120 min at room temperature, then frozen in dry ice–ethanol and either immediately defrosted under warm tap water or stored at -70° for later use. After warming, the emulsion was shaken for 60–240 min at room temperature. The fixed specimens were incubated in 5% β -mercaptoethanol, 1% Triton-X100, 0.1 M Tris pH 7.4 for 24 h at 37° (ref. 21). The preparation was washed extensively with PBS plus 0.5% Triton-X100 (PBST), suspended in 0.1 M Tris pH 7.4, 1 mM CaCl₂, 0.1% Triton X-100 and 1,000 U ml⁻¹ Collagenase VII (Sigma), and incubated at 37° for 24–48 h²¹. The preparation was then washed extensively with PBST and pre-incubated for a few hours at room temperature with 1% bovine serum albumin (BSA) in PBST. The fixed specimens were incubated with affinity-purified anti-*lin-14* antibody, diluted 1:10 times in PBST, 1% BSA, incubated for 24 h at room temperature, and washed for 4–24 h at room temperature with PBST, 1% BSA. The sample was then incubated at room temperature for 4–24 h with FITC-coupled goat anti-rabbit antibody (Cappel) at $10 \mu\text{g ml}^{-1}$ that had been pre-incubated at room temperature for 4–24 h with fixed *C. elegans* specimens to remove any cross-reacting antibodies. After extensive washing in PBST, 1% BSA, the specimens were mounted on a 4% low-melting point agarose pad with $5 \mu\text{l}$ of $1 \mu\text{M}$ DAPI and 0.5% *n*-propyl gallate in 70% glycerol–30% 0.1 M Tris pH 9.0. Fluorescent nuclei were visualized on a Zeiss universal microscope using Zeiss filter sets 487710 (FITC) or 487705 (DAPI) and a Planapo 63 objective. Kodak TriX400 black and white film or Kodak TMY 5053 film or Fuji 400 colour film 'pushed' two f-stops in development were used for photography. No immunofluorescent staining was observed in control experiments using affinity-purified sera from pre-immunized rabbits, using the anti-*lin-14* antibodies pre-incubated with excess *lacZ*–*lin-14* fusion protein, or using no primary anti-*lin-14* antibody before the secondary antibody staining (data not shown). Pre-incubation of the anti-*lin-14* antibodies with excess β -galactosidase and *E. coli* protein extracts did not affect the immunostaining (data not shown).





the *lin-14* protein (data not shown). Differentiated neuronal, muscle, and hypodermal cells accumulate the *lin-14* protein at these late stages but are not known to be affected by the gain-of-function *lin-14* mutations. As in the case of wild-type, because these cells do not divide, cell-fate changes associated with these *lin-14* mutations could not be scored⁶. Thus, the gain-of-function *lin-14* mutations cause inappropriate expression or accumulation of the *lin-14* protein at late developmental stages and destroy the normal *lin-14* temporal regulation (Fig. 4).

Molecular basis of *lin-14* cell fate specification

The nuclear localization of the *lin-14* protein suggests that it regulates the pattern of gene expression of the cells that accumulate it either transcriptionally, by binding to DNA adjacent to 'downstream' genes^{12,13}, or by controlling the RNA processing of other genes¹⁴. Candidate 'downstream' genes whose expression may be controlled by *lin-14* include the *lin-14* gene itself, other heterochronic genes, such as *lin-28* and *lin-29*⁶ and cell-type and temporally regulated genes such as the vitellogenin genes¹⁵. The *lin-14* nuclear protein could either activate early larval stage-specific genes or repress late larval and adult stage-specific genes. DNA sequence analysis of the *lin-14* gene has revealed no homologies to any other gene (T. Bürglin, J. Gatto and G.R., unpublished observations).

The particular early or late cell fate specified by the level of *lin-14* gene activity is distinct for many of the post-embryonic blast cells affected by *lin-14* mutations⁶. Presumably, the *lin-14* temporal specification signal is interpreted in each nucleus in the context of cell-specific differences caused by differing position, lineage and so on. These differences may be reflected in the distinct spectra of previously specified or partitioned nuclear factors that interact in combination with the *lin-14* nuclear protein to cause a distinct response in nearly every cell.

Temporal control of the cell lineage

We have shown that the temporal pattern of the *C. elegans* post-embryonic cell lineage is generated, in part, by the temporal regulation of *lin-14* protein levels. During wild-type development, the *lin-14* protein level peaks early during L1 in the hypodermal (Fig. 4) and intestinal lineages, later during L1 in many neurons, and during L3 in Pn.p cells. Gain-of-function *lin-14* mutations dramatically alter this temporal regulation; the high level of the *lin-14* protein normally observed only in the nuclei of embryos and L1 stage animals is inappropriately maintained during all of post-embryonic development in these strains (Fig. 4). These data indicate that the *lin-14* gain-of-function mutants reiterate L1-specific lineages in the hypodermal and intestinal cell lineages⁶ because the inappropriate presence of high levels of *lin-14* protein in nuclei at late developmental times repeatedly specifies L1 blast cell fates. Conversely, the absence of the *lin-14* protein in strains bearing *lin-14* loss-of-function mutations specifies precocious stage-specific cell fates during each larval stage. Thus, both the high and low levels of the *lin-14* protein are instructive (Fig. 4).

These data indicate that in the hypodermal and intestinal cells the normal temporal regulation of the *lin-14* protein level specifies the time when a switch between L1-specific and L2-specific cell fates takes place. This occurs when cells with less than a particular threshold level of *lin-14* protein execute L2-specific cell lineages rather than L1-specific lineages. Some *lin-14* alleles, however, do not affect the time of appearance of L1-specific and L2-specific patterns of division, but cause precocious appearance of L3-specific, L4-specific, and adult cell lineages only⁷. These alleles show that the *lin-14* gene can specify a later cell fate independently from the earlier fates, indicating that either intermediate levels of the *lin-14* protein, or separate *lin-14* gene products or regulatory regions, specify these distinct

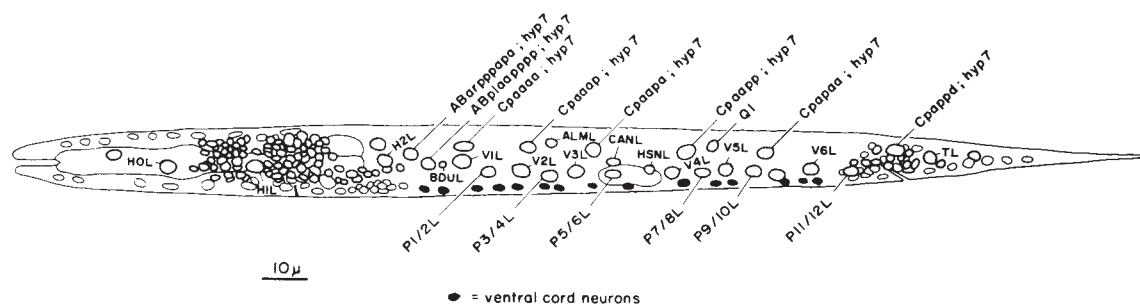
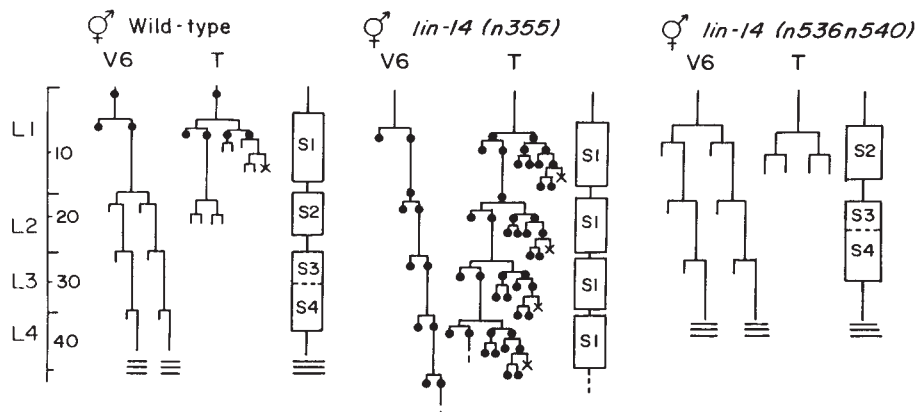


FIG. 3 Arrangement of neuronal and hypodermal nuclei on the left side of a newly hatched L1 (from ref. 10 as corrected by C. Loer, D. Waring, C. Kenyon and J. Sulston, personal communication). Cells which do not divide post-

embryonically are named by their lineage, for example, Cpaapp. Post-embryonic blast cells are renamed H1, H2 and so on. Nuclei which were difficult to identify, such as the nerve ring, are unnamed in this figure.

FIG. 4 The *lin-14* protein temporal gradient in wild-type and the perturbations of this gradient in *lin-14* mutants. Shown are two examples of cell lineages from the hermaphrodite lateral hypodermis that are affected by *lin-14* mutations. The wild-type lineages of blast cells V6 and T are shown in the left panel. Altered lineages of these blast cells in the *lin-14* gain-of-function mutant *n355* are shown in the middle panel. Altered lineages in the *lin-14* loss-of-function mutant *n536n540* are shown to the right. The cells that accumulate the *lin-14* protein during development are marked by a dot; those that do not accumulate *lin-14* protein are unmarked. In wild-type *C. elegans* the blast cells V6 and T accumulate the *lin-14* protein during early larval stage one (L1), but after the L1-specific cell divisions, the *lin-14* protein staining in the daughter cells and their descendants fade. No nuclear staining is visible in these lineages during L2, L3, L4, adult stages. The normal temporal progression of these cell lineages is summarized to the right of this panel by the sequence S1, S2, S3, S4, and the three horizontal lines representing the adult differentiation of these cells⁶. In the gain-of-function *lin-14* mutant *n355* the normal diminution of *lin-14* protein levels in V6 and T progeny does not occur. Thus,



these strains do not generate the normal *lin-14* protein temporal gradient so that at late developmental times cells do not switch from their early fate. These cells all reiterate the early S1 fate. In the loss-of-function *lin-14* mutant *n536n540*, no detectable *lin-14* protein at any stage is detected. These cell lineages precociously express the later larval cell lineages S2, S3, S4, adult.

temporal fates. If intermediate levels of the *lin-14* protein specify particular temporal fates, then the *lin-14* temporal switch may not be binary but in fact graded.

The generation of this temporal switch must depend both on properties of the *lin-14* gene and protein and on interactions with other gene products. The *lin-14* gain-of-function mutations disrupt this regulation. These gain-of-function mutations have been shown to delete sequences from the 3' end of the *lin-14* mRNA and must remove a negative regulatory sequence that normally mediates the down-regulation of the *lin-14* protein level during development⁸.

Other heterochronic genes could control developmental timing of the *C. elegans* cell lineage by participating in the generation or interpretation of the *lin-14* temporal switch. For example, a recessive mutation in the gene *lin-4* causes reiterations of early larval cell lineages equivalent to those of *lin-14* gain-of-function mutations^{5,6}, and requires a functional *lin-14* gene for this phenotype (V. Ambros, personal communication). The *lin-4* gene product may interact with the negative regulatory site that is deleted in the gain-of-function *lin-14* mutants. Other heterochronic mutations in *lin-28*, *lin-40*, and *lin-41* cause precocious expression of late larval and adult cell lineages⁶ (V. Ambros, personal communication). By monitoring the nature of the *lin-14* protein gradient in strains bearing mutations in these other heterochronic genes, we will be able to assess whether these genes are upstream or downstream of *lin-14* in this regulatory pathway.

Thus, the level of *lin-14* protein within nuclei decreases over time like a molecular hour glass to specify developmental time to the cell lineages affected by *lin-14* mutations. These data show that the normal temporal progression of at least some of

the cell fates observed during *C. elegans* post-embryonic development is not an emergent property of the dynamics of other developmental decisions taking place, but is under explicit control of the heterochronic genes.

The *lin-14* gene is not the only developmental clock. *Lin-14* mutant animals continue the moulting cycle, even though the fates expressed at each stage are inappropriate⁶. In addition, the maturation of the germ line occurs at normal times in these mutants⁸. These processes may be regulated by other temporal control genes.

Pattern-formation genes and evolution

Many of the developmental control genes so far identified seem to constitute components of binary or multistate switches like *lin-14*¹. It is the spatial and temporal asymmetries in the patterns of developmental control gene activity during ontogeny that make cells or sets of cells different from each other. Mutations such as the *lin-14* gain-of-function and loss-of-function mutations, which change the expression pattern of such control genes, would lead to significant morphological change and could be the main cause of the variation necessary for evolutionary change. Indeed, the *C. elegans* heterochronic mutations are analogous to the heterochronic variation between species noted in phylogenetic studies^{6,16}. This heterochronic variation could be due to mutation in one or a few heterochronic genes like *lin-14*, rather than many mutations that incidentally change developmental timing. More generally, mutations that change the spatial, temporal or cellular asymmetries in pattern formation gene activities may be the underlying cause of the manifold forms of metazoans and may be a significant force in evolutionary change. □

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LETTERS TO NATURE

Does supernova 1987A contain a rapidly vibrating neutron star?

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If the recently reported^{1,2} 0.5-ms-period pulsed optical signal from the direction of supernova 1987A originated in a young neutron star, its interpretation as a rotational period has difficulties. The surface magnetic field would have to be much lower than expected, and the high rotation rate may rule out preferred nuclear equations of state^{3,4}. Here we point out that a remnant radial vibration of a neutron star, excited in the supernova event, may survive for several years with about the observed (gravitationally red-shifted) period. Heavy ions at the low-density stellar surface, periodically shocked by the vibration, may efficiently produce narrow pulses of optical cyclotron radiation in a surface field of $\sim 10^{12}$ gauss. These pulses may be modulated only slightly by a

much slower stellar rotation because of the nearly isotropic emission mechanism and smearing from the strong gravitational bending of light rays^{5,6}. We do not attempt to explain the reported¹ 8-hour modulation, which may be a result of timing noise (ref. 7 and J. Katz, preprint).

Present upper bounds on the luminosity of SN1987A limit the surface magnetic field of a neutron star rotating twice a millisecond to no more than 10^9 gauss. Unless the field rises to 10^{12} gauss in $\sim 10^3$ yr, by the emergence of a presently buried field or from magnetothermal generation⁸, such a low field marks this event as very different from the Crab supernova as well as from those explosions responsible for the half-dozen other pulsar/supernova remnant associations. Moreover, the inferred rotation rate, maintained without instability, may place too severe a constraint on the equation of state of nuclear matter^{3,4}.

Neutron-star vibrations have been discussed at some length in the literature^{9–11}. From dimensional considerations, the fundamental radial mode period P is expected to be of the order of $(G\rho)^{-1/2} \sim 10^{-4}$ s, with ρ the mean neutron-star density. The observed period of 5×10^{-4} s is close to that for a typical neutron-star model^{10,11} (corrected for the gravitational redshift) with mass $M \approx 1M_\odot$ and radius $R \approx 10^6$ cm. Non-radial and higher-

order radial modes would be damped on timescales of ≤ 1 yr by gravitational, neutrino and electromagnetic radiation^{9,10,12}. A major damping source for the fundamental radial mode is the URCA neutrino-emission process¹³, which gives a damping time of $\sim 10^2$ yr or longer, depending on the amplitude. However, this timescale could be reduced dramatically by several effects. (1) 'Exotic' interior states with enhanced weak interactions would magnify the radial vibration damping. These include central π -condensates or quark matter^{14,15}. Confirmation of our model may rule out the presence of these in the putative neutron star produced by SN1987A, unless the neutrino emission which they can generate is suppressed by superfluid energy gaps. (2) Gravitational radiation emission would increase if there were coupling to non-radial vibrations. Such a coupling will arise when the underlying spherical symmetry is broken, for example if the neutron star is rotating. For a neutron star with a uniform density, the rotation-dependent gravitational radiation damping time is¹⁶ $\sim 2 \times 10^3 P^4$ yr, where P is the rotation period in seconds. Our model would then require a slowly rotating neutron star, with $P \geq 0.1$ s. With such a period, a 10^{12} -gauss field does not cause the neutron star spindown power to exceed the current supernova luminosity. (3) Hydrodynamic eddy diffusion and electron viscosity in the core would probably damp out an initially very large amplitude of vibration that existed when the star was still very hot.

The optical radiation is expected to originate in a region no larger than a light-travel size of 150 km. For an optical pulse luminosity of $\sim 10^{36}$ erg s⁻¹ (18th magnitude^{1,2} at a distance of 55 kpc) originating near a neutron-star surface, the brightness temperature $T \geq 10^{15}$ K implies either a typical radiating-particle energy $\geq 10^2$ GeV or coherent optical emission. X-ray measurements of the supernova luminosity¹⁷ require the total power radiated by the pulsar into the supernova remnant not to exceed that emitted into the optical band by more than about 10^3 . Incoherent curvature radiation into the optical band is so inefficient that such an emission mechanism places a generally intolerable burden on any magnetosphere acceleration model. If electron synchrotron radiation is the source of optical emission, the local magnetic field B must certainly be much less than 10^{10} G. Even with a much lower field it may be difficult to accommodate such intense optical electron synchrotron radiation. For electrons of energy E to radiate at a peak photon energy ϵ (eV), we require

$$B \sin \alpha \leq 2 \times 10^5 \left(\frac{10 \text{ MeV}}{E} \right)^2 \epsilon \text{ gauss} \quad (1)$$

where α is the electron pitch angle. A synchrotron radiation spectrum (intensity $\sim \epsilon^{1/2}$) could give $(3 \text{ eV}/\epsilon)^{1/2} \geq 10^{-3}$ of its energy in the optical band if the peak photon energy $\epsilon \leq 10^6$ eV. For $E \geq 10^{11}$ eV and such a value of ϵ ,

$$B \sin \alpha \leq 2 \times 10^3 \text{ gauss} \quad (2)$$

This extreme constraint on B suggests that the radiation arises

instead from cyclotron radiation from heavy stellar surface ions, Fe^{Z+} for example. These can produce optical cyclotron radiation with a typical photon energy of

$$\epsilon \approx 3 B_{12} \frac{2Z}{A} \text{ eV} \quad (3)$$

where B_{12} is the magnetic field in units of 10^{12} gauss and A is the atomic number of the ion. Fully stripped energetic but nonrelativistic Fe ions can give strong cyclotron optical emission in a field $B \approx 10^{12}$ gauss. We propose that these ions may be given the required velocities ($v \approx 10^{10}$ cm s⁻¹ for 10^2 -GeV ^{26}Fe ions) after the radial vibration steepens into a strong shock when it reaches the very small densities and scale heights near the stellar surface¹⁸. The shock speeds up, and ion velocity behind the shock increases as density drops sharply at the surface. In the absence of detailed numerical analysis we are unable to specify the shock details. Just after the shock the kinetic energy carried by ions will initially dominate that carried by electrons. If the local post-shock density is $\leq 10^{-3}$ g cm⁻³, optical synchrotron radiation from the shocked Fe ions can compete favourably with collisional loss to the much less energetic electrons around them.

Because of the short travel time for the shock passing through the surface of the neutron star, and the short ion cyclotron lifetime ($\leq 10^{-5}$ s), a sharp pulse may be expected within each cycle. Furthermore, because the emission is concentrated around the ion gyration frequency, the vibration-shocked surface can produce a reasonably efficient conversion of internal vibration energy to optical radiation.

The total luminosity of SN1987A sets a lower limit to the neutron-star rotation period of $\geq 20 B_{12} L_{38}^{-1}$ ms, where L_{38} is the supernova luminosity in 10^{38} erg s⁻¹. As noted above, our model requires $P \geq 0.1$ s. As the cyclotron emission occurs in a magnetic field that varies over the surface of the star, modulation at the stellar rotation period is expected. The amplitude of this modulation may be rather small because of the isotropic energy input from the vibration, the fairly isotropic geometry of cyclotron emission, and the strong gravitational bending of the emitted light rays^{3,6}. However, the polarization of the emitted optical light will also vary with the stellar rotation and should be detected more easily.

Future period observations should severely test the predictions of our model; specifically the period of the neutron-star vibration (unlike that from rotation) should not increase significantly with time (although the luminosity will decrease as the vibration is damped); and the rotation period of the star should be found to be ≥ 0.1 s unless the vibration has been excited recently. The frequency corresponding to the peak emission in the SN1987A optical pulsar spectrum could be used to estimate the magnetic field at the surface of the neutron star (see equation (3)). The detection of X-rays from the neutron star before the vibration dies out would provide another clue as to the origin of the optical light. $\square$

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Was the millisecond pulsar in SN1987A spun up or born spinning fast?

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THE discovery¹ of an optical pulsar in SN1987A with a period of 1,968.629 Hz raises many interesting issues, chief among them being the question of how the pulsar came to acquire such a rapid rotation rate. An obvious but possibly incorrect assumption is that it was simply born that way owing to a large specific angular momentum in the iron core that collapsed. Here we argue that this millisecond pulsar, like others observed previously, has been spun up by accretion. In this case the accreted angular momentum comes from the mixed mantle and helium core of the ejecta, of which roughly $0.1 M_{\odot}$ fell back during the first day after the explosion. This sizeable mass, and hence angular momentum, of the re-imploded material is at least partly a consequence of the blue supergiant nature of the progenitor star.

The evolution of the helium core of a massive star should, in the later stages, be decoupled from the envelope structure of the star. One expects, therefore, that the explosion of SN1987A was very similar, except in aspects related directly to the radius of its hydrogen envelope, to a typical type II supernova. There is considerable evidence to suggest that SN1987A was not particularly unusual; this evidence comes from the neutrino burst, the nucleosynthesis, the bolometric light curve and the late-time spectrum from γ -ray to infrared frequencies. But we have never before seen within any young supernova remnant a pulsar having such a rapid rotation rate. Could it be that this one is in a process of rapid deceleration from a (commonly occurring) initial state of high angular momentum? The limits placed on such deceleration by the discovery observation in SN1987A suggest that it is not: observations over a 7-h period (broken into half-hour intervals) revealed no more than a 0.001-Hz drift in the frequency, so that the period derivative $\dot{P}$ is at present less than 10^{-14} . The limits are in fact even more constraining, because the variations fit a sine wave with amplitude 0.0015 Hz to better than 5%, but we will adopt the less stringent number for now. Thus $P/\dot{P}$ is considerably greater than 1,000 yr and, unless $\dot{P}$ increases markedly in the future, the pulsar will retain its millisecond period for a long time to come. Observations of pulsars in young supernova remnants, and pulsar statistics, admittedly of objects far older than 1987A, suggest that pulsars initially have periods of 0.01–0.5 s and magnetic fields of $\sim 10^{12}$ gauss with a small spread^{2–4}. Such a large magnetic moment is precluded for the SN1987A pulsar because it would then be an extraordinarily bright object, much brighter than the observed brightness of roughly 100 times that of the Crab nebula pulsar in the energy band sampled⁵. Why, then, does the SN1987A pulsar possess such unusual properties?

We begin by noting that there is plentiful angular momentum in the cores and mantles of massive stars. The newly discovered pulsar has an angular momentum of $\sim 10^{49}$ erg s; even without invoking differential rotation, a B0 star on the main sequence (of mass $17 M_{\odot}$) with a typical rotation period of 2 days (ref. 6) and a radius of $8 R_{\odot}$ would have angular momentum of several times 10^{52} erg s. As the star evolves through the stages of nuclear burning—burning hydrogen, helium, carbon, neon, oxygen and silicon in the core and shell—convection, in particular, transports angular momentum out of the central regions of the star. The transport coefficient for this process is unknown, as is the initial distribution of angular momentum inside the star. Thus

the star might or might not end up with a collapsing core that was rotating so fast as to be near breakup.

The iron core that collapses experiences more stages of convection than any other part of the star. Each time the core contracts to ignite a new fuel, these convective episodes sweep angular momentum out, usually to a region just inside the Chandrasekhar mass. Convective shells, especially oxygen-burning, may be efficient in carrying the angular momentum out still farther. Thus a relatively slowly rotating core of $1.5 M_{\odot}$ could plausibly collapse within a surrounding mantle endowed with considerable angular momentum, especially near its inner edge. A very approximate estimate for the angular momentum contained within the $2.0 M_{\odot}$ of the core at central carbon ignition is 2×10^{50} erg s (Fig. 7 of ref. 7; see also ref. 8). Later stages of convective carbon, neon, oxygen and silicon burning would transport angular momentum but would leave a substantial residual in the $\sim 1 M_{\odot}$ of material outside the iron core. About $0.1 M_{\odot}$ of this material could easily contain an angular momentum of 10^{49} erg s.

Moreover, SN1987A has clearly demonstrated the importance of mixing. The composition of the heavy-element core has been stirred by Rayleigh–Taylor instabilities, some in the reverse shock as the helium core runs into its own hydrogen envelope, some powered by the decay of radioactive ^{56}Ni and ^{56}Co , and possibly some caused by the unknown explosion mechanism itself (which may involve neutrinos creating a sizeable bubble of radiation inside the star⁹). In this mixing process, angular momentum as well as composition will inevitably be distributed, and in particular some material with high specific angular momentum will be mixed inwards¹⁰.

Once the explosion has occurred and the shock wave is propagating outwards, a substantial amount of matter may fall back onto the neutron star during the reverse shock into the mantle, caused by the helium core running into the massive hydrogen envelope^{10–13}. Before the discovery of the pulsar, it was estimated that $\sim 0.1 M_{\odot}$ of material had fallen back in SN1987A, although the errors of the calculation permit the actual value to be several times greater (or smaller). Thus sufficient angular momentum could fall back to spin up the neutron star.

The accretion is sensitive to the density of material in the vicinity of the neutron star, which decreases with time roughly as t^{-3} until the reverse shock arrives and slows the expansion. For a red supergiant, a more typical supernova precursor, it takes ten times longer for the helium core to encounter a mass equal to its own, so the reverse shock is delayed. Meanwhile the density decreases and less matter accretes. It is estimated¹⁰ that 100 times less matter would accrete in the explosion of a red supergiant than in SN1987A. This could explain why neutron stars born in more typical type II supernovae end up rotating more slowly. If so, the rapidly spinning pulsar may be yet another special attribute of the relatively small radius of the anomalously blue supergiant progenitor.

The limit to the degree of spin-up, however, lies not just in the supply of angular momentum but in the efficiency with which it can be added. We assume that material can accrete with a tangential velocity no greater than the Keplerian orbit speed. General relativity modifies the newtonian value¹⁰ because there are no stable orbits inside 3 Schwarzschild radii, R_s (for a rapidly rotating neutron star this value is reduced to 1.75 – $2.2 R_s$ (ref. 14)). The angular momentum delivered to a (non-rotating) neutron star of mass $1.5 M_{\odot}$ is 4.6×10^{48} erg s per $0.1 M_{\odot}$ accreted ($3^{1/2} R_s c$ per g; (ref. 14)). This means that roughly $0.2 M_{\odot}$ needs to have fallen back onto the neutron star. If the neutron star were still rotating highly differentially, with the crust moving much faster than the superfluid central core (which contains most of the moment of inertia), the required mass could be much less. Recent studies²⁵ suggest, however, that the crust and core are efficiently coupled and that the timescale for communicating angular momentum is quite short, certainly less

than a year. If the timescale were one year, $\dot{P}$ would be much greater than the limit given above. For now we adopt the more restrictive assumption of rigid rotation.

The (baryon) mass of the neutron star inferred from the neutrino burst is in the range $1.2\text{--}1.7 M_{\odot}$ (ref. 16) with most probable values near the middle of the range; clearly, an addition of $0.2 M_{\odot}$ to a $1.5\text{--}M_{\odot}$ neutron star would give a mass of $1.7 M_{\odot}$. The maximum mass of a hypothetical neutron star^{17,18} having equation of state 'F' (which is relatively 'soft') and a period of 0.5 ms is $1.87 M_{\odot}$ (for which the moment of inertia is $1.16 \times 10^{49}\text{ erg s}$). Without rotation, a neutron star of mass $1.7 M_{\odot}$ would be unstable to collapse for such an equation of state. Although the gravitational masses (roughly 10% less than the baryon mass) of neutron stars in our Galaxy may be less than $1.5 M_{\odot}$ (ref. 19), the new pulsar probably needs to be more massive than this in order to rotate as rapidly as it does^{17,18}. Accretion, as described here, would produce the required mass increase naturally.

Most of this mass addition would have occurred early on, with a maximum rate occurring soon after the reverse shock reached the core (that is, 2 hours after the explosion²⁰). Most of the accretion energy would have been expelled in the form of neutrinos²¹. And additional energy radiated would be negligible compared to the supernova energy on day 1. Although our model has a large accretion rate in the early phases, this can be reduced by the escape of radiation from the vicinity of the neutron star after about 7 months (ref. 10). The present accretion rate onto the pulsar must be very small so as to accommodate the known bolometric luminosity of $3 \times 10^{38}\text{ erg s}$ (R. Catchpole, personal communication). All radiation with energy below 10 keV would still be effectively converted to light in the optical and infrared bands²², so the present accretion rate is below about $10^{-8} M_{\odot}\text{ yr}^{-1}$. For such a small $\dot{M}$ (and $\dot{P}$), the pulsar is in no immediate danger of becoming a black hole. If added at the Keplerian velocity, accretion at a rate of $10^{-8} M_{\odot}\text{ yr}^{-1}$ would contribute angular momentum to the neutron star at a rate several orders of magnitude below the limit inferred from $\dot{P}$ in the observations.

The mass addition could also have the interesting effect of reducing the surface magnetic field strength, as a result of an initially very high accretion rate of material with a disordered magnetic moment, which could bury the magnetic field of the accreting neutron star. A field of 10^{12} gauss would be overwhelmed by an accretion rate of only $0.002 M_{\odot}$ per day, which is considerably less than the rate expected. Alternatively, convection and nuclear activity in the accreted material may have reduced the surface field²³. An interesting consequence of the model proposed above is that the magnetic dipole field of the core, initially buried by accretion, may eventually emerge, leading to a brightening of the pulsar and a more rapid spin-down.

There are surprisingly few major observational differences between a pulsar born rotating fast and one spun up during the first day of its life. The latter would certainly have a greater mass, but not so great as to be incompatible with the proposed nuclear equation of state. To distinguish between the two may require a consideration of all observations and models of the supernova, paying special attention to the explosion mechanism. Our model offers the advantage of explaining why this pulsar is unusual and of coupling this peculiarity to the structure of the progenitor star Sk-69 202. Given that a plausible explanation for the blue nature of that star is, at least in part, a manifestation of the low metallicity of the Large Magellanic Cloud^{20,24}, one implication of the model is that similar millisecond pulsars may have been created in other metal-deficient regions. However, there are other factors besides metallicity that are involved in the evolution of a blue supergiant progenitor²⁴. These include the mass of the star ($15 \leq M/M_{\odot} \leq 22$), the efficiency of semi-convection (which is small), and the amount of mass loss (also small). It is unlikely that all pulsars in the LMC would be born

as millisecond pulsars, but it is possible that some could. Pulsar 0540-69, for example, which is also in the LMC and within a remnant of estimated age 800 yr (ref. 25), has a 'normal' period of 50 ms and probably also a strong magnetic field. $\square$

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Geminga and the search for optical counterparts of γ -ray-burst sources

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THE nature of gamma-ray-burst (GRB) sources has remained a mystery, in part because of the lack of any optical identification. Recently, deep CCD photometry has identified the optical counterpart G" to the γ -ray source Geminga, whose location on a colour-magnitude diagram is unique¹. Although the X-rays from G" are probably due to thermal emission from the neutron star, the implied column density ($\geq 5 \times 10^{20}$) is inconsistent with the distance ($D < 100\text{ pc}$) required to fit the optical flux to the same spectral component. Here I show that the optical emission could be due instead to a cold accretion disk with accretion rate $\dot{M} \approx 10^{11}\text{ g s}^{-1}$. This has promising implications for the search for the optical counterparts to GRB sources whose basic physical parameters may resemble those of radio pulsars and related objects such as Geminga. I argue that a similar search in the field of a GRB location should produce candidates similar to G" if $20\text{ pc} \leq D_{\text{GRB}} \leq 500\text{ pc}$, as predicted by the disk-reprocessing model for the associated optical transients. This possibility is discussed in the light of the fact that the first optical counterpart to a GRB source may already have been found, which, if it is confirmed, may be used to test the accretion-disk model.

The confirmed optical identification of 1E0630+178 with a 25-mag blue object (G") makes it very likely that this X-ray source is an isolated neutron star and strongly suggests that it is the counterpart to the high-energy γ -ray source Geminga¹. The detection of thermal emission from a neutron star is not new. The discovery of G" is significant, however, because of its

similarity to Vela-type pulsars and because of the possibilities implied in respect of the search for the quiescent counterparts of GRB sources, which may themselves be isolated, magnetized neutron stars.

Although the basic mechanism for producing GRBs is not yet well understood, support for the neutron-star model is extensive² and has been augmented by the confirmation of low-energy features (interpreted as cyclotron resonances) in some GRB spectra³⁻⁸. It is not surprising, therefore, that GRB sources have sometimes been associated with extinct pulsars⁹⁻¹¹. The similarities between the two include the following. (1) Both seem to be isolated neutron stars¹²⁻¹⁴. (2) At least some of the GRB sources, like pulsars, are highly magnetized (with surface magnetic fields $\geq 10^{12}$ gauss (refs 5, 6)). (3) Several pulsars (including PSR0531+25 and 0833-45) emit detectable fluxes of γ -rays up to energies in excess of 2×10^3 MeV (ref. 15), with a flux density that falls off as a power law characterized by an index $\alpha \approx -1$. Significantly, the Gamma-Ray Spectrometer on the Solar Maximum Mission satellite has discovered that emission of >1 MeV (with $-2 \leq \alpha \leq 0$) is a common component of GRBs, and that in many cases it actually dominates the emitted power^{6,16}. (We note, however, that GRB spectra below ~ 1 MeV are not always similar to those of rotation-powered pulsars.) (4) There is some evidence that GRB sources may have periods¹⁷ similar to those of pulsars¹⁸. However, the pulsar period distribution peaks around ~ 0.5 s (and extends down to 1.6 ms in the case of PSR1937+214), whereas the periods in GRBs (if confirmed) fall in the range ~ 1 –10 s. Although this distinction may be simply a selection effect (that is, faint, slow pulsars are undetectable at large distances, and GRB periods $\ll 1$ s may be 'hidden' by the intrinsic rapid variability of the burst flux), it might also be an indication that GRB sources are indeed extinct pulsars and that the transition from one population to the next occurs when the spin period of the neutron star exceeds ~ 2 s (ref. 11). On the other hand, it might simply indicate that although the two types of systems have similar properties, they form by different processes which lead naturally to different period distributions¹⁹.

Is it possible then, in view of this close analogy between Geminga-type sources and GRB sources, to predict the observable characteristics of the optical counterparts to the latter? Before addressing this question, let us consider the status of

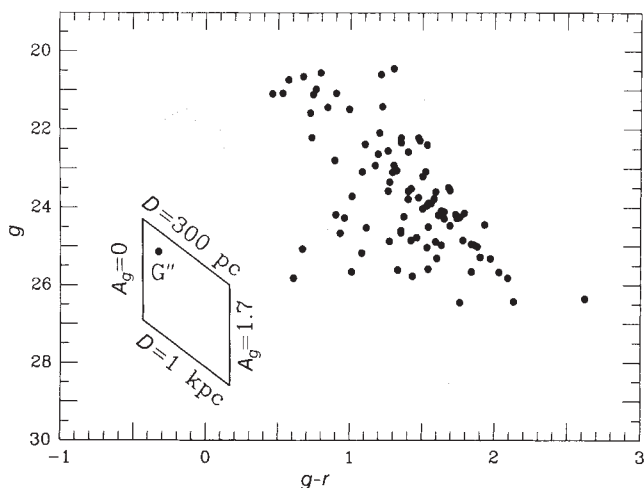


FIG. 1 Colour-magnitude diagram for the stars in the field of the Einstein error circle for the X-ray source 1E0630+178 (Geminga). At a distance $300 \text{ pc} \leq D \leq 1 \text{ kpc}$, thermal emission from the surface of a neutron star with a temperature ($\sim 1.1 \times 10^6 \text{ K}$) fitted to the X-ray data cannot account for the optical flux. The region enclosed by the diamond corresponds to the magnitude and colour of an accretion disk with accretion rate $\dot{M} \approx 10^{11} \text{ g s}^{-1}$. A_g is the reddening in the direction of this source. (Data are taken from ref. 1.)

Geminga and its identification with G'' . Deep CCD photometry^{1,20} at the Einstein Observatory shows that the star in question is by far the bluest object in the field of the Einstein error circle of the X-ray source 1E0630+178 (Fig. 1). Even though deep optical searches reaching beyond 25 mag reveal many objects, a source such as G'' , with a large and significant departure from colours expected and observed from the field stars of similar magnitude, occupies a distinct, outlying position on a colour-magnitude diagram. Quantitative photometry and reanalysis of the Einstein data led Halpern and Tytler¹ to conclude that the optical and X-ray emission together are consistent with a single-temperature black-body spectrum of temperature $T = (4.3 \pm 1.7) \times 10^5 \text{ K}$, although the X-ray data alone are best fitted by $T = 1.1 \times 10^6 \text{ K}$. This is an important distinction because the lower temperature implies a distance $D < 100 \text{ pc}$, whereas the higher temperature requires both that $D < 2 \text{ kpc}$ and that the optical emission must have another source. In view of the fact that the column density N_H corresponding to the single-temperature fit is at least 5×10^{20} (which is much larger than the measured values within 100 pc of the Sun in this direction), a line of sight with this value of N_H would more probably extend to $\sim 500 \text{ pc}$. There is thus an inherent difficulty with the single-temperature model. (One should keep in mind, however, that the surface composition of isolated neutron stars is poorly known and a black-body fit to the X-ray spectrum may not be adequate²¹.) A second objection to the smaller distance implied by the single-temperature fit is the correspondingly small displacement of the source away from our galactic plane ($\leq 7 \text{ pc}$), which is statistically unlikely¹.

The optical emission from G'' may be due to a distinct (perhaps synchrotron¹) component. I propose that at least part of this optical flux may be emitted by a cold accretion disk surrounding the neutron star. Systems such as this have already been considered in disk models for pulsar action²², and as possible γ -ray-burst sources^{10,23,24}. In addition, there is some evidence that these disks are necessary to account for the optical transients associated with some GRBs^{14,25,26}.

To explore this possibility, I have assumed a model in which the optical and X-ray fluxes are due to a thermal, isotropically radiating neutron star²⁷ with a temperature $T_0 = 1.1 \times 10^6 \text{ K}$, mass $M = 1.4 M_\odot$ and radius $R = 10 \text{ km}$, and a cold disk whose emission may be treated as a sum of black bodies^{6,14}. The maximum disk temperature, $T_{\text{max}} = [3GM\dot{M}/65\pi\sigma R^3]^{1/4} = 1.22 \times 10^5 (M/1.4 M_\odot)^{1/4} (\dot{M}/10^{10} \text{ g s}^{-1})^{1/4} (R/10 \text{ km})^{-3/4} \eta^{-3/4} \text{ K}$, is attained at the equatorial radius $R_{\text{max}} = (49/36)R_{\text{in}}$, where $R_{\text{in}} \equiv \eta R$ defines the inner edge of the disk and η (~ 3 for the systems considered here^{6,14}) is a parameter characterizing the Alfvén radius. Thus, accretion disks driven by ordinary degenerate electron viscosity have temperatures (corresponding to accretion rates $\dot{M} \approx 10^{10} \text{ g s}^{-1}$) that are too low for them to be detected from X-ray emission, but that permit them to contribute significantly to the optical flux because of their large emitting surface area.

Figure 1 shows the magnitude and colour expected of G'' , together with the published data¹. Because $\eta \approx 3$, only the parameter $\dot{M}$ can influence the result significantly. (The inclination angle i of the disk to the line of sight enters only through a factor of $\cos i$, which I will assume to be 0.7 in all the calculations.) For a fixed $\dot{M}$, the range in the g magnitude reflects the uncertainty in the source distance D , whereas the extent in $g-r$ on the colour-magnitude diagram corresponds to the unknown degree of reddening A_g . (The value $A_g = 1.7$ is the maximum observed in this direction¹.) Once the neutron-star temperature and distance ($\sim 500 \text{ pc}$) are fixed by the X-ray data, the optical data, which provide a colour and magnitude, can be fitted with just one essential parameter ($\dot{M}$). The diamond-shaped region shown in Fig. 1 corresponds to an accretion rate $\dot{M} = 10^{11} \text{ g s}^{-1}$. Note that, for these parameter values, a distance $D \approx 500 \text{ pc}$ would imply an amount of reddening corresponding to $A_g \sim 0.3$ – 0.4 .

The success of this fit is promising in so far as GRB sources are concerned because they may contain cold accretion disks^{10,14,23-26}. Application of the disk-reprocessing model to the optical transients associated with GRBs^{14,19} predicts distances in the range ~ 20 –500 pc for the four known optical-transient (OT) GRB sources, not unlike that of Geminga. Of these, the OT1928/GRB1978 object²⁸ is predicted by this model to be the closest, with $20 \text{ pc} \leq D \leq 150 \text{ pc}$. Perhaps not coincidentally, this also happens to be the GRB source for which the best attempts have been made to search for a quiescent optical counterpart^{29,30}.

The colour-magnitude data for all those sources in the field of the OT1928 error box for which colour information is available³⁰ are shown in Fig. 2. Although these sources are much fewer in number than those in Fig. 1, the similarity of the two plots is nonetheless apparent. Also shown in this figure is the range in B and $B-V$ predicted on the basis of the simple neutron-star-plus-disk model described above. In this case, the amount of reddening is expected to be very small ($A_B \approx 0$) because the source is relatively close. The allowed range in B and $B-V$ is instead due to the uncertainty in the accretion rate and distance. As before, standard parameter values were chosen for the neutron star, which was also assumed to have a surface temperature $T_0 \approx 10^5 \text{ K}$, consistent with the most constraining X-ray observations currently available for this source³¹.

As was the case for G'', there is no question that the source DD is unique among the objects detected in the field of the OT1928 error box: main-sequence stars with $B \geq 25$ and $B-V \leq 0$ would have to be early types with $D \approx 10 \text{ kpc}$, which is unlikely given that the galactic latitude of this field is $\sim -84^\circ$, and active galactic nuclei with this colour are typically much brighter ($V < 20$). CC has a magnitude similar to DD, but its colour and magnitude together are consistent with a normal late-K-type main-sequence star. The proximity of DD to the predicted range of B and $B-V$ suggests that, if this object is indeed an isolated neutron star surrounded by an accretion disk, then $10^7 \text{ g s}^{-1} \leq \dot{M} \leq 10^9 \text{ g s}^{-1}$ and $D \approx 100 \text{ pc}$, as predicted by the disk-reprocessing model¹⁴. Thus, in conjunction with deep CCD photometry to identify more precisely the magnitude and colour of DD, confirmation of candidate DD as the burst source would strongly support this model, whereas ruling it out would argue against it. If this model is correct and the connection between GRB sources and pulsars is borne out by further observational

testing, the longer periods (if confirmed), the lower neutron-star surface temperatures and the apparently lower accretion rates in GRB sources argue either for an older (perhaps extinct) branch of the pulsar population or for two distinct populations differing only in the scale of the physical parameters characterizing their constituents.

Obtaining CCD photometry to a limiting magnitude (5σ detection threshold) of 26 in B , V and R band-passes is feasible in principle at prime focus on a 4-m telescope. Although the historical OTs (found on archival photographs) and the modern-day GRBs are separated by as much as 40 years, the sources will not have shifted position by more than $\sim 9''$ for a transverse velocity of $\leq 50 \text{ km s}^{-1}$ at a distance of 200 pc. Because of its unique colour, any GRB source within the CCD's $\sim 4' \times 4'$ field of view centred on the OT location should stand out clearly in a colour-magnitude diagram. Such an effort should at least be carried out for the source DD, which is arguably our strongest candidate for an optical counterpart to a GRB source. $\square$

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Evidence from Voyager II photometry for early resurfacing of Umbriel

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THE uranian satellite Umbriel's dark, heavily cratered surface is remarkable for its apparent uniformity in Voyager II images¹. Its most conspicuous geological feature is a comparatively high-albedo, annulus-shaped deposit which covers the floor of the 40-km diameter crater Wunda¹. Here we present new Voyager II albedo maps of Umbriel which reveal that its surface is subdivided into low-contrast, crudely polygonal areas ranging in size from tens to hundreds of kilometres (Fig. 1). The largest polygons are elongate with systematically trending northeast-southwest boundaries. Some of the polygonal areas form topographic depressions several

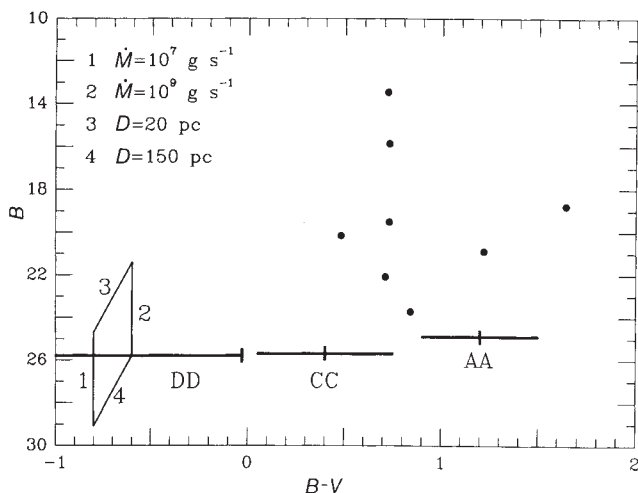


FIG. 2 Colour-magnitude diagram for the stars in the field of the 1928 optical transient, which seems to be associated with the 1978 November 19 γ -ray burst. As is the case for G'', the position of source DD is unique. Its proximity to the region of B versus $B-V$ predicted by the 'single-neutron-star plus cold-disk' model makes it a very strong candidate for the optical counterpart to this GRB source. (Data are taken from ref. 30.)

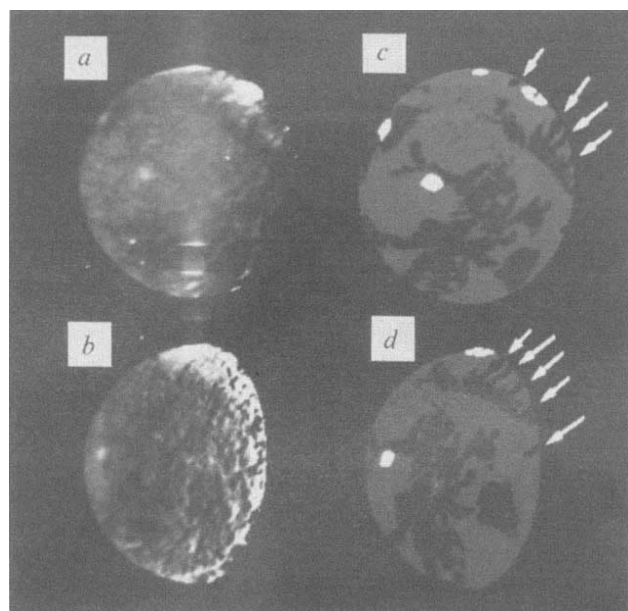


FIG. 1 *a*, Voyager image FDS 26825.51 after shading correction and contrast enhancement. Phase angle is 31° and resolution is 16 km per line pair. *b*, Highest-resolution Voyager image (FDS 26840.04), processed as in *a*. Phase angle is 56° and resolution is 8 km per line pair. *c*, *d*, Sketch maps showing locations of features identified in our albedo map (Fig. 2*a*) as they would appear in images at left. Arrows identify the locations of parallel topographic grooves. The brightest features are bright craters. Irregularly shaped dark areas are prominent dark polygonal terrains.

kilometres in depth. We suggest that this newly discovered global albedo pattern is a relic of the early tectonic disruption of Umbriel's surface.

We have prepared a new, photometrically accurate map of Umbriel's surface which reveals a low-contrast global albedo pattern not recognized previously (Fig. 2*a*). This map is a composite constructed by first correcting the brightnesses in each Voyager image to compensate for differences in viewing and illumination geometry (Fig. 1*a*, *b*), and then projecting the image into standard polar stereographic coordinates. The digital map in Fig. 2*a* represents a mosaic of four such projected images (Voyager FDS 26788.15, 26797.22, 26825.51, 26840.04).

To perform shading corrections to each image, we applied the analytical equation of Hapke²⁻⁴, which describes the variation of surface brightness of rough particulate surfaces with changing photometric geometry. For any given surface element, photometric geometry is specified by three angles: incidence angle, i , emission angle, ϵ , and phase angle, α (the angle subtended between two vectors pointing from the surface to the Sun and to the observer). Hapke's equation requires five model parameters whose values we determined from observations of Umbriel's changing brightness during different phases of the Voyager II encounter. Two of the parameters, $\tilde{\omega}_0$, the average particle single-scattering albedo, and g , the asymmetry factor in the Henyey-Greenstein⁵ particle-phase function, are closely related to the composition and mechanical structure of soil particles. The opposition effect (a surge in brightness observed in particulate surfaces at low illumination and small viewing angles) is described by two parameters: h , which relates the angular width of the surge to the state of compaction of surface particles, and B_0 , which describes the amplitude of the opposition surge. Macroscopic surface roughness is described by Hapke's $\bar{\Theta}$ parameter, the average slope angle of sub-resolution-scale topographic relief.

Preliminary global-average values of these parameters have been determined from 'clear-filter' ($\sim 0.48 \mu\text{m}$) observations by Voyager of Umbriel's integrated-disk brightness⁵. That study

provides the best available estimates of opposition surge parameters ($B_0 = 2.01$, $h = 0.06$), but improved estimates of $\tilde{\omega}_0$, g and especially $\bar{\Theta}$ can be obtained on the basis of Umbriel's disk-resolved photometric behaviour. Accordingly, we have collected disk-resolved measurements of Umbriel's brightness from five clear-filter Voyager images (FDS 26788.15, 26797.22, 26825.51, 26840.04, 26904.04) covering phase angles from 10.4° to 142.9° and averaged in bins of 5° of photometric latitude and longitude over each visible disk (excluding the few visible bright craters). Optimal values of $\tilde{\omega}_0 = 0.35$, $g = -0.18$ and $\bar{\Theta} = 26^\circ$ were then determined by fitting Hapke's equation to these data using a nonlinear least-squares algorithm⁶.

Figure 1*a* and *b* shows the highest-resolution images of Umbriel after performing the shading correction and after contrast stretching to enhance details. Quasi-polygonal discontinuities between coherent regions of lower and higher albedo can be identified clearly in Fig. 1*a* and, less easily, in Fig. 1*b* (for which resolved topographic texture more strongly obscures albedo markings). The fact that these features become more distinct with decreasing phase angle suggests that they are indeed albedo markings rather than shadows cast by topography. Some of the discontinuities define closed, approximately polygonal areas (Fig. 1*c*, *d*), ranging from less than 50 km to greater than 600 km in size. In many cases, albedo contrast between adjacent polygonal areas is too variable along the boundaries to allow clear definition of complete polygons.

In Table 1 we list normal albedos (the reflectance at normal incidence and emission angles) of selected features on Umbriel. The average normal albedo of Umbriel is 0.21. The brightest feature on the satellite, the bright annulus crater, has an albedo of 0.49 and a contrast of 40%. In comparison, the apparent albedo contrast of probable bright-crater ejecta is only about 5%. The photometric contrast between the darkest polygons and typical surrounding regions is only 2.4%.

The composite albedo map can be used to examine the distribution of dark polygons over Umbriel's visible surface. On scales of less than about 100 km, polygons seem to have random orientations. However, sub-global-scale polygons at 25°S , 300°E and 73°S , 270°E are elongated, with nearly parallel northeast-southwest-trending lengthwise boundaries. A pattern of parallel topographic grooves (shown by arrows in Fig. 1*c* and *d*) cross-cuts the long boundary of one of these polygons at large angles.

At the highest resolution possible for Voyager (11 km per line pair), it is difficult to identify visually any possible topographic boundaries that may be separating polygons. Thomas⁷, however, has recently derived accurate elevation profiles along the limb of Voyager image FDS 26840.04, which projects into our albedo map along the arrow labelled A-A' in Fig. 2*a*. In Fig. 2*b* and *c*, we compare the limb elevation profile of Thomas with the corresponding normal albedos along the length of the limb projection in Fig. 2*a*. A close, but not perfect, correspondence between albedo and topography is evident: areas of lower albedo are topographically lower, and higher albedos are associated with topographic highs. We stress that the topography shown

TABLE 1 Clear-filter normal albedos for selected features on Umbriel

Feature	Location	Normal albedo
Global average		0.21 ± 0.02
Darker polygons	$19^\circ\text{S } 310^\circ\text{E}$	0.202 ± 0.011
	$65^\circ\text{S } 7^\circ\text{E}$	0.191 ± 0.004
Brighter polygons	$83^\circ\text{S } 135^\circ\text{E}$	0.204 ± 0.002
	$83^\circ\text{S } 98^\circ\text{E}$	0.209 ± 0.002
	$56^\circ\text{S } 257^\circ\text{E}$	0.202 ± 0.002
Bright craters	$82^\circ\text{S } 270^\circ\text{E}$	0.232 ± 0.007
	$42^\circ\text{S } 192^\circ\text{E}$	0.226 ± 0.004
Bright annulus	$5^\circ\text{S } 273^\circ\text{E}$	0.491 ± 0.051

was determined from limb geometry, that is, by a method totally independent of photometry (which is to say, of albedo).

There is no obvious stratigraphic relationship between darker and brighter polygons; but their existence, along with systematic patterns of global orientation and the nature of the topography, is consistent with the interpretation that they are relics of tectonic breakup and resurfacing of Umbriel. The tendency for lowest-albedo materials to occur in low-lying terrains is consistent with an internally driven resurfacing event involving darker materials. Such darker materials are known to have been extruded on other uranian satellites, that is, certainly on Miranda and probably on Oberon.

The apparent muting of topographic boundaries between polygons by accumulated impact craters suggests that the proposed resurfacing occurred very early in Umbriel's geological history. Indeed, Strom⁸ has suggested, on the basis of cratering statistics, that Umbriel was resurfaced earlier than other uranian satellites. However, any geological interpretation of the global albedo pattern discovered in our study must be seen in the light

of the very low spatial resolution of the Voyager images of Umbriel. A global quasi-polygonal, low-contrast albedo pattern definitely exists on Umbriel. Limited topographic data suggest that a correlation between albedo and topography exists. The geological interpretation of these patterns is uncertain. □

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Influence of long-range transport of combustion emissions on the chemical variability of the background atmosphere

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MAUNA Loa Observatory, located 3,400 m above sea level on the island of Hawaii in the middle of the Pacific Ocean, is a critical site for determining the background chemical reactivity of the unpolluted atmosphere and for monitoring its rate of change on a global scale. However, recent measurements of soluble nitrogen (principally HNO_3) at the observatory find mixing ratios rising from their expected background values of 0.02–0.03 parts per 10^9 by volume (p.p.b.v.) in the winter to 0.07–0.12 p.p.b.v. in late summer with three-hour events as high as 0.25 p.p.b.v. (ref. 1). This raises the specific question of contamination by the long-range transport of pollution¹ and a broader question of the chemical variability of the background atmosphere. Here we show that a general circulation transport model which simulates the global spread and deposition of emissions from fossil-fuel combustion can reproduce the nitrogen measurements at the Mauna Loa Observatory. By isolating individual source regions, we show that US emissions are responsible for the late summer increase and that Asian emissions cause a smaller increase in the spring. These simulations, together with the earlier observations, indicate frequent contamination of the Mauna Loa Observatory by the long-range transport of reactive trace gases, such as HNO_3 , and suggest a highly variable background atmosphere. It is essential that we are aware of such variability in order to discern anthropogenic effects on the atmosphere.

We use a global transport model which has already demonstrated the important role of dry deposition in acid deposition over North America² and the impact of combustion nitrogen on the global nitrogen budget³. The model has 11 altitude levels (31.4, 22.3, 18.8, 15.5, 12.0, 8.7, 5.5, 3.1, 1.5, 0.5 and 0.08 km), a horizontal grid size of ~265 km, and a time step of ~26 min (ref. 4). Using the 6-h time-average winds and precipitation provided by a parent general circulation model^{5,6}, the collection of gaseous and particulate reactive nitrogen compounds that result from the combustion emissions are transported as a single tracer, NO_y . We have already discussed the formulation of tracer transport^{3,4}, the global source of nitrogen resulting from fossil-fuel combustion³, the effective dry deposition of NO_y ^{3,7} and the effective removal of NO_y by precipitation^{3,4}.

Those emissions not deposited in the source region become

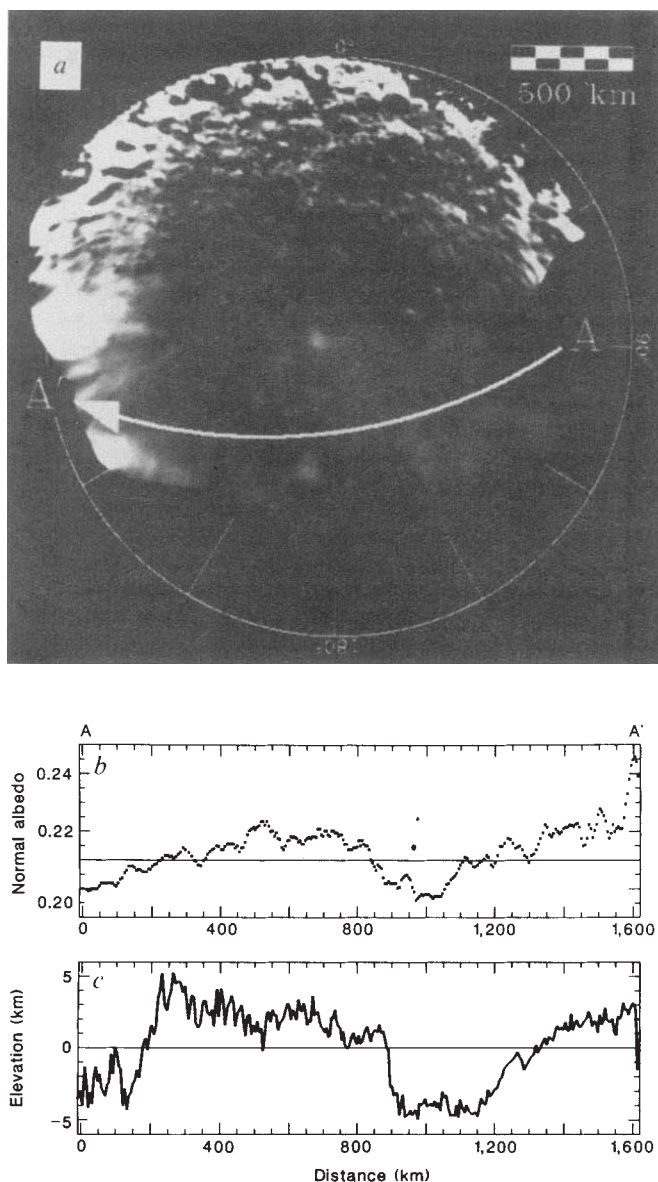


FIG. 2 a, Composite polar stereographic projection of four Voyager images of Umbriel. Arrow A–A' identifies the projected location of the limb from image FDS 26840.04 (Fig. 1b). b, Plot of normal albedos from a, along section A–A'. c, Elevation profile along section A–A', corresponding to albedo plot in b.

available for long-range transport. This available NO_y is specified by basing the model's dry deposition and precipitation removal on measurements of nitrogen deposition in precipitation and of surface concentrations of individual nitrogen species. A single linear parameter is adjusted to bring the model's yearly integral of precipitation removal over North America into agreement with observation². This same parameter leads to a good simulation of the yearly wet deposition of nitrogen over the European source region and remote locations in the Northern Hemisphere³. Dry deposition uses effective deposition velocities for NO_y , which are based on the surface concentrations of the individual reactive nitrogen species measured over the United States.

The collective transport and parameterized deposition of NO_y precludes the explicit transport of individual nitrogen compounds, particularly insoluble species, such as peroxyacetyl nitrate (PAN) and other organic nitrates. However, we find that much of the transport from Asia and North America to the central Pacific is in stable subsiding air with little precipitation, and that the simulated NO_y deposition and surface concentrations in the central Pacific agree reasonably with observations³. The controlling factor is the model's meteorology—which both lifts the NO_y into the free troposphere (that region of the troposphere that is not directly influenced by boundary-layer mixing) and transports it to Hawaii—and not the detailed chemistry in the source region and along the transport path.

In Fig. 1, we compare the measurements of soluble nitrogen at the Mauna Loa Observatory¹ with the simulated monthly average mixing ratios of NO_y in grid boxes located over Hawaii. The model's simulation of NO_y at 940-mbar level in the atmosphere, ~0.5 km above sea level, reproduces both the magnitude and seasonal variation of the observations from the Mauna Loa Observatory. In contrast, the simulated mixing ratios at the 685-mbar level, approximately the height of the Mauna Loa Observatory, agree only with the low winter levels and show no increase in the other seasons. The model does not have sufficient horizontal and vertical resolution to simulate the island of Hawaii, let alone the two mountain peaks and the local complex flow. It simulates long-range transport to grid boxes in the vicinity of Hawaii, and the 685-mbar grid box represents the free troposphere over the Pacific rather than the Mauna Loa Observatory.

Furthermore, extremely high-resolution (10 km × 10 km) simulations of the air flow around Hawaii show that the normal winds at 3,400 m in the free troposphere are greatly modified by the mountainous terrain⁹. Air parcels are carried up from below in direct flow from the windward side of the mountain and also in return flow from eddies that form in the lee of the island. As a result, the air sampled at the Mauna Loa observatory is a complex mixture of normal free-tropospheric air found at 3,400 m, air from stable layers below 3,400 m and above the maritime boundary layer, air from the maritime boundary layer (that is, below the maritime inversion layer and above the sea surface) and air from the island's boundary layer, which is likely to be contaminated by local pollution and natural emissions. Coincident measurements of humidity, wind direction and condensation-nuclei concentration are used to delete soluble nitrogen observations that may be contaminated by local pollution and natural emissions from both the island and the sea¹, but measurements in air from stable layers below 3,400 m are retained. Over the ocean, the model produces a stable layer above a weakly mixed maritime boundary layer that is ~1.5 km thick with a well mixed lowest level of ~0.2 km. This structure is in qualitative agreement with the observed maritime boundary layer over the eastern tropical Pacific⁸, although the observed thickness increases from ~0.5 km near the Baja to 1.5–2.0 km near Hawaii.

By running separate integrations for the global, Asian and US sources, we are able to identify the individual contributions. The observed summer mixing-ratio maximum, which is well

simulated in the model at 940 mbar, results from a sharp increase in transport from the United States to Hawaii that continues through the autumn. During the winter and spring, nitrogen emissions from Asia dominate and produce the spring increase. The low background levels at the 685-mbar level, with a hint of a spring maximum, are completely dominated by emissions from Asia, with little contribution from the United States or the rest of the globe.

A more complete picture of the atmospheric transport is provided by the simulated time series of NO_y at the 940-mbar level over Hawaii in Fig. 2. We see a collection of discrete transport events superimposed, at a frequency of 3–5 days, over a very low background. In particular, we see that the summer maximum is the result of a few large events. The limited observations appear to have a similar character (B. J. Huebert, personal communication). Both this simulation and the observations at Mauna Loa Observatory suggest that the levels of reactive gases in the remote atmosphere may be highly variable.

During the summer, in both the model and the real atmosphere, the time-mean Pacific subtropical high moves from a latitude of ~20° N off the Baja to a position north of Hawaii at ~35° N (ref. 10). At times, this produces a subsiding flow in the lower troposphere from the south-west United States and Mexico to Hawaii. When combined with surface transport from the source regions and small-scale vertical mixing over arid land, episodic transport of NO_y to the vicinity of Hawaii occurs in the model. Three-dimensional trajectories and a detailed

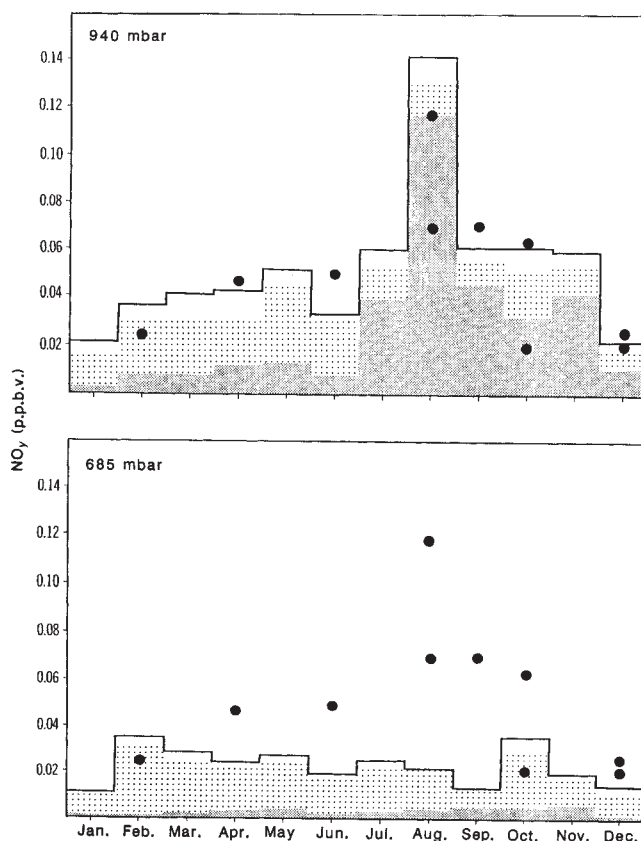


FIG. 1 Ten-day averages of soluble-nitrogen measurements at Mauna Loa Observatory compared with simulated monthly average mixing ratios of NO_y in the 940-mbar and 685-mbar grid boxes located over Hawaii. The dark shaded portion represents the simulated contribution of the US source, and the lighter shading represents the contribution from Asia (in our model a source region bounded on the south by the Equator, on the north by 60° N latitude, on the west by 50° E longitude and on the east by 150° E longitude) and the white area under the solid line represents the contribution from the rest of the globe, principally Mexico, Central America and Europe. The observations are represented by large black dots.

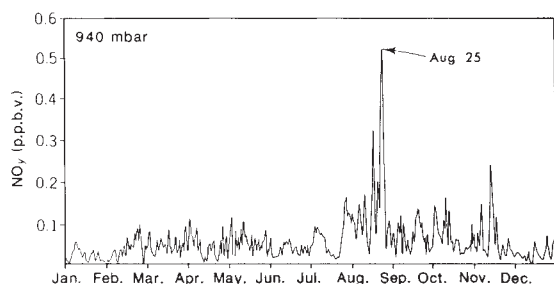


FIG. 2 The NO_y time series at the 940-mbar level in the vicinity of Hawaii constructed with instantaneous values taken every six hours from the simulation for a global source of combustion nitrogen.

synoptic analysis were used to identify this complex transport process (W.J.M., manuscript in preparation).

When using observed wind data, vertical velocity is not available and one must approximate atmospheric motion by assuming that either pressure or potential temperature remains constant. Such approaches can be misleading. Robinson and Harris¹¹ use observed winds to calculate approximate trajectories on constant-pressure surfaces for a number of actual transport events at Mauna Loa Observatory in August. Finding that none of the 700-mbar back-trajectories from Hawaii returned to the United States and none of the 850-mbar trajectories from the United States approached Hawaii, they conclude that US emissions are not involved¹¹. We believe that the actual summer-time transport events are as complex as those simulated by the model and cannot be represented by trajectories on constant-pressure surfaces.

The transport of NO_y from Asia is composed of a large number of relatively small (~ 0.1 p.p.b.v.) events. Asian dust, which also has surface sources and is removed by precipitation, may serve as a surrogate for Asian emissions. The dust has been observed throughout the central Pacific, including Mauna Loa, and has a spring maximum^{12,13}. Nitrogen emissions from Asia are expected to follow a similar transport path. To reach Hawaii in the subtropics, the Asian emissions must first be lifted into the free troposphere by storms and carried eastward and north-eastward over the central Pacific. Then the NO_y must be caught in descending air, transported anticyclonically (clockwise) towards the Equator and carried far enough south to be caught in the low-level easterly flow. Such paths have been determined for actual springtime Asian dust events¹⁴ and for our simulated springtime transport of Asian NO_y . Both the lifting over Asia and the descent over the Pacific occur at a range of levels in the troposphere. As a result, the model's Asian emissions are dispersed throughout the lower troposphere. In contrast, the transport from North America is descending all the way to Hawaii in the stable flow of the Pacific subtropical high and should arrive just above the maritime boundary layer.

Based on these results, other model studies⁹, observations^{12,13} and actual climatology¹⁰, we conclude that the maximum in soluble nitrogen observed at Mauna Loa Observatory in the summer and autumn is a result of US fossil-fuel combustion. It travels to Hawaii in stable descending air and is carried up to the observatory by the complex flow associated with the mountain orography. Springtime measurements in the vicinity of Hawaii, but away from the influence of the island's orography, should find episodes of elevated NO_y (mainly from Asia) up into the middle troposphere, while summertime measurements should find episodes of elevated NO_y concentrated in shallow layers just above the maritime boundary layer. □

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Synthesis of bulk superconducting $\text{YBa}_2\text{Cu}_4\text{O}_8$ at one atmosphere oxygen pressure

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THE well-known 90-K superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$ ('123') is the first ($n=0$) member of a homologous series of compounds with the general formula $\text{Y}_2\text{Ba}_4\text{Cu}_{6+n}\text{O}_{14+n}$. These compounds combine layers of copper–oxygen pyramids with single and/or double copper–oxygen chains. The $n=2$ member, $\text{YBa}_2\text{Cu}_4\text{O}_8$ ('124'), with double Cu–O chains, and the $n=1$ ytterbium analogue, $\text{Yb}_2\text{Ba}_4\text{Cu}_7\text{O}_{15}$ ('247'), which mixes single and double chains, were first observed as intergrowths in bulk 123 by electron microscopy^{1,11}. The 124 phase was then synthesized as a majority phase in thin films^{2,3,12}, and its crystal structure was determined⁴ and found to be in agreement with the model proposed from microscopy. An important advance in the synthesis of bulk materials, the result of extensive pressure–temperature phase equilibria studies, was the isolation of 124 and 247 at oxygen pressures of >200 atm, and detailed determinations of their crystal structures (see, for example, refs 5–9). High-pressure studies have also shown that the 124 phase could be made with many rare-earth elements¹³. Here we report the synthesis of the 124 phase in pure bulk form by a novel synthetic route in a flowing oxygen stream at 1 atm pressure. This technique allows $\text{YBa}_2\text{Cu}_4\text{O}_8$ to be synthesized without specialized equipment, will make it generally available for study of its normal and superconducting properties, and will make possible more extensive comparisons with $\text{YBa}_2\text{Cu}_3\text{O}_7$ and other high- T_c superconductors. Our results suggest that 124 is the thermodynamically stable phase at low temperatures and 1 atm pressure, under oxidizing conditions; the usual inability to synthesize it in these conditions is probably due to the limitations of reaction kinetics.

The synthesis is a two-step process. In the first step, starting materials are mixed in the correct stoichiometric proportion and heated very slowly to 750 °C in dense, oversized Al_2O_3 crucibles. They are then allowed to react for 16–24 h. All heating, soaking and cooling is carried out in flowing O_2 . Best results are obtained when an intermediate mixing-and-grinding step is performed after the first few hours of reaction at 750 °C. Different combinations of starting materials yield slightly different results. We investigated three different sets of starting materials: (1) $\text{Y}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$, $\text{Ba}(\text{NO}_3)_2$ and $\text{Cu}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$; (2) $\text{Y}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$, $\text{Ba}(\text{NO}_3)_2$ and CuO ; and (3) Y_2O_3 , $\text{Ba}(\text{NO}_3)_2$ and CuO . The first of these, with all materials present as nitrates, always yielded the most phase-pure materials, although 124 did occur as the major phase for all three sets of starting materials. As it was necessary to use hydrated nitrates, the amount of water in the starting materials was determined directly by weight loss on decomposition to the simple oxides, which indicated that

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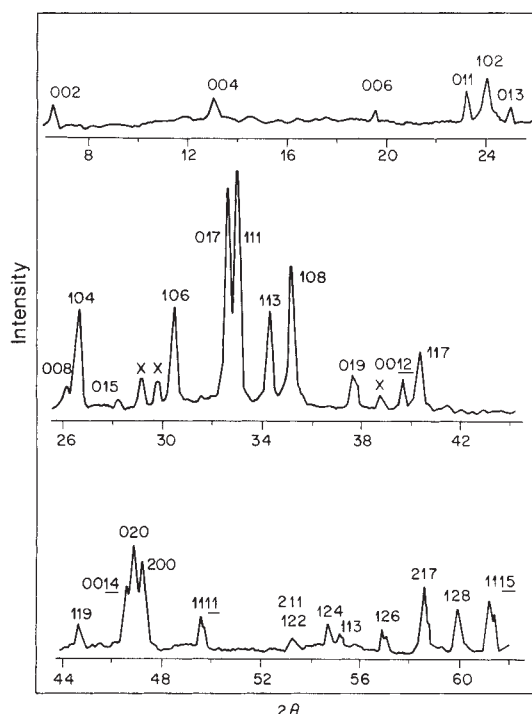


FIG. 1 Powder X-ray diffraction pattern of $\text{YBa}_2\text{Cu}_4\text{O}_8$ prepared using a sodium carbonate catalyst. Peaks are indexed on an A-centred orthorhombic cell with lattice parameters $a = 3.846$, $b = 3.871$, $c = 27.262$ Å. Impurity peaks are marked with crosses.

our starting materials were $\text{Y}(\text{NO}_3)_3 \cdot 5.8\text{H}_2\text{O}$ and $\text{Cu}(\text{NO}_3)_2 \cdot 3.3\text{H}_2\text{O}$.

In the second step, the pre-reacted powder, after grinding, is mixed with an approximately equal volume of alkali carbonate powder. The alkali carbonate acts to catalytically enhance the reaction rate. For the synthesis of 124, either Na_2CO_3 or K_2CO_3 can be used for this purpose, but Na_2CO_3 yields better phase purity. The 124-carbonate mixture is ground, placed in silver-foil packets or crucibles and heated at 800°C in flowing O_2 for 3 days. The carbonates are not molten at this temperature. $\text{YBa}_2\text{Cu}_4\text{O}_8$ is the main phase present after just one day, but the phase purity improves for longer reaction times. If the first reaction step is not carried out, BaCO_3 forms in the initial stages of the carbonate reaction and the desired phase cannot be made. The 124 phase can also be made, albeit less reliably, at 825°C ; but by 850°C , $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ forms as a major impurity phase, which is consistent with the metastability of $\text{YBa}_2\text{Cu}_4\text{O}_8$ described in ref. 8. We are able to make 124 as the majority phase at temperatures as low as 700°C by this process. At the lowest temperatures, approximately equal mixtures of K_2CO_3 and Na_2CO_3 function as the most effective catalysts, as such a mixture is closer to its melting point during the reaction than is either of the pure carbonates. After reaction, the products are washed briefly (15 min) in water to remove excess alkali carbonate and dried by gentle heating in air. The product is a fine-grained powder.

A representative powder X-ray diffraction pattern of the material produced by this technique is shown in Fig. 1. The peaks have been indexed by position and by the intensities calculated from the published crystal structure. The materials made by this method, although in general not perfectly single-phase, are at least as phase-pure as materials prepared at high oxygen pressures. Three impurity peaks are present in Fig. 1 with intensities of $\sim 10\%$ of the main peak intensity, at d values (inter-planar spacings) of 2.83 Å, 2.92 Å and 2.32 Å (CuO). For the Na_2CO_3 -catalysed formation of 124 at 800°C , the 123 phase is not usually present as an impurity, but it may be present if K_2CO_3 is used as the catalyst. Improved phase purity may be

achieved by careful control of the composition and uniformity of the hydrated-nitrate starting materials, or of the initial stages of the dehydration process, which involves the evolution of a large amount of gas from a mixture which is apparently molten at very low temperatures (100 – 200°C). Least-squares refinement of the crystallographic unit cell from 17 of the powder diffraction peaks between 2θ values of 30° and 62° , consistent with an A-centred orthorhombic cell, yields lattice parameters $a = 3.8457(9)$, $b = 3.8711(9)$ and $c = 27.2616(61)$ Å, in excellent agreement with the orthorhombic cell parameters published previously⁹.

Figure 2 shows the results of d.c. magnetization measurements on $\text{YBa}_2\text{Cu}_4\text{O}_8$ prepared at 800°C in O_2 with Na_2CO_3 as catalyst. The sample is a pellet pressed from the fine-powder product, but not sintered. The measurements were made using an SHE squid magnetometer in a field of 25 Oe. Flux expulsion below T_c (on field-cooling) corresponds to $\sim 60\%$ of that expected for a perfect diamagnet. This is a large apparent superconducting volume fraction for a pressed-powder sample, and actually represents a conservative lower limit for the true fraction of superconducting material, because flux trapping in pores may in general limit the flux expulsion significantly, as is evident here in the slowly increasing diamagnetism measured at temperatures well below the superconducting transition. The transition temperature (77 K) is within the range of temperatures reported previously but is somewhat lower than the maximum of 80 – 81 K reported for $\text{YBa}_2\text{Cu}_4\text{O}_8$ prepared at high oxygen pressures. The reason for this variation is not yet clear, as the compound has been reported to be stoichiometric in composition for all components, including oxygen⁵. Annealing our material at 450°C in flowing O_2 for 16 h did not increase T_c substantially. There is no diamagnetism above 77 K, indicating, as did the powder X-ray diffraction data, that $\text{YBa}_2\text{Cu}_3\text{O}_7$ is not present as an impurity under these synthetic conditions.

We have also measured the temperature dependence of the crystallographic cell parameters between 30 and 320 K. We were particularly interested to see whether structural anomalies occur near 240 K, where kinks in the resistance and Hall coefficient have been observed in thin-film samples (ref. 10 and S. Martin *et al.*, manuscript in preparation). The temperature dependence was measured by taking θ – 2θ scans at moderately high angles (56 – $63^\circ 2\theta$) and fitting to the positions of six peaks of the powder pattern. The instrumental resolution was ~ 0.028 Å^{–1} full width at half maximum. The results of the fits (Fig. 3) do not indicate any anomalies in the lattice parameters in the range 30 – 320 K. In addition, we observed no indication of a lower symmetry in

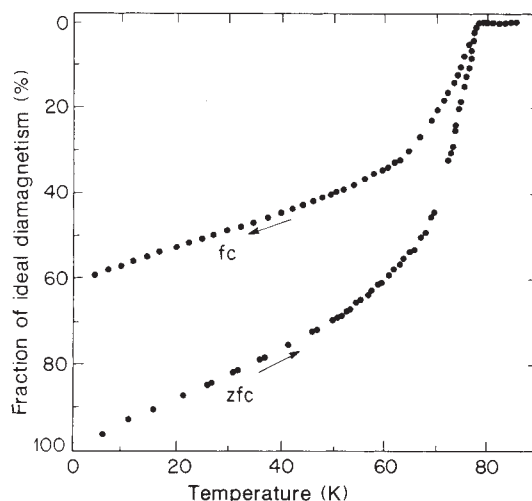


FIG. 2 Temperature dependence of d.c. magnetization for a pressed-powder compact of $\text{YBa}_2\text{Cu}_4\text{O}_8$, measured in a d.c. field of 25 Oe. Zero-field cooled (zfc) and field-cooled (fc) measurements are shown.

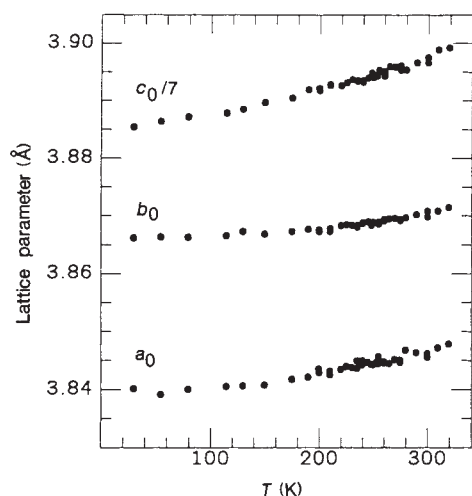


FIG. 3 Temperature dependence of the crystallographic unit-cell parameters for $\text{YBa}_2\text{Cu}_4\text{O}_8$ between 30 and 320 K.

the powder scans at 30 K, nor were extra peaks observed that would indicate a multiplication of the unit-cell volume.

In conclusion, we have shown that the compound $\text{YBa}_2\text{Cu}_4\text{O}_8$ can be prepared as a bulk phase at ambient pressure in a novel multi-step process which employs an alkali carbonate to enhance the reaction rate. Special care must be taken in the selection of the starting materials, the order of the reaction steps, selection of the container materials, the temperature of reaction, and the use of a non-detrimental reaction-rate enhancer. The process described here yields high-quality 124 without specialized equipment and will allow considerably more research to be performed on its physical properties. Finally, we note that our attempts to synthesize $\text{Y}_2\text{Ba}_4\text{Cu}_7\text{O}_{15}$ by the same method were not successful. □

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Separation of paramagnetic and diamagnetic molecules using high- T_c superconducting ceramics

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WITH the advent of high-temperature superconductivity, the search is on for applications of these materials. Here we outline a new method for the separation of paramagnetic and diamagnetic molecules using the repulsive interaction between a magnetic dipole and a micro-cavity in a ceramic material which is in the supercon-

TABLE 1 O_2/N_2 ratio in out-flow gas through $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ and a $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet as a function of temperature

$\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ pellet		$\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet	
O_2/N_2	T (K)	O_2/N_2	T (K)
0.04	85.3	0.26	84.8
0.05	85.6	0.27	85.0
0.05	86.2	0.26	85.2
0.05	86.4	0.27	85.6
0.08	86.6	0.27	86.2
0.10	87.6	0.27	87.5

ducting state. We present experiments of the permeation of air through $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ and through $\text{Bi}_{1.8}\text{Pb}_{0.2}\text{Ca}_2\text{Sr}_2\text{Cu}_3\text{O}_x$ ceramic pellets. These experiments show the preferential permeability of nitrogen compared with that of oxygen in the diamagnetic state of the superconductors below the critical transition temperature, T_c . This effect is quenched by the application of a large enough external magnetic field. As far as we know it is the first application of the porosity of the ceramic superconductors.

The energy of interaction (E) of a magnetic dipole (m) and a superconducting wall is easily calculated by the mirror principle¹ (see Fig. 1). This interaction is given by:

$$E = \frac{\bar{m}_1 \bar{m}_2}{r^3} - \frac{3(\bar{m}_1 \bar{r})(\bar{m}_2 \bar{r})}{r^5} = \frac{m^2}{r^3} \{\cos \varepsilon - 3 \cos \theta_1 \cos \theta_2\} \quad (1)$$

where $\bar{m}_2$ is the mirror image of $\bar{m}_1$, and $\theta_1 + \theta_2 = 180^\circ$. The thermal tumbling of $\bar{m}$, when averaged over all orientations θ , gives $\langle E \rangle = \frac{3}{2}(m^2/r^3)$. Suppose we enclose the tumbling magnetic dipole in the centre of a cubic cavity: at what distance from the walls of the cavity is the repulsive interaction of the superconducting walls equal to the thermal kinetic energy of the elementary magnetic moment? We have $6\langle E \rangle = \frac{3}{2}kT$, and thus $r = (6m^2/kT)^{1/3}$. We can now substitute m^2 by the paramagnetic susceptibility $\chi(T) = m^2 N/3kT$, where N is Avogadro's number, so that

$$r = \left(\frac{18\chi(T)}{N} \right)^{1/3} \quad (2)$$

From equation (2) we can estimate the effective distance r at which the repulsive interaction between the molecular magnetic moment and the superconducting diamagnetic enclosure will strongly affect the thermal diffusivity of the magnetic dipole. Let us compare the relevant size parameters for oxygen (a paramagnetic molecule) to those of diamagnetic nitrogen. The effective molecular diameter of oxygen, as derived from the Einstein-Stokes equation using the diffusion constant of O_2 in ethanol², is 1.67 Å, and the same calculation for N_2 in carbon tetrachloride yields 1.24 Å; in comparison, $2r$ for oxygen (using $\chi(91 \text{ K}) = 1.08 \times 10^{-2} \text{ ml mol}^{-1}$ (ref. 3)) is 1.37 Å. Nitrogen, on the other hand, is diamagnetic, $\chi(300 \text{ K}) = -12 \times 10^{-6} \text{ ml mol}^{-1}$. We see that the diameter, $2r$, is comparable with the effective diameter as calculated from the Einstein-Stokes equation. The Pauling dimensions for O_2 are 2.8 Å in width and 3.9 Å in length, with a kinetic diameter $\sigma = 3.46$ Å. The corresponding values for nitrogen are 3.0 Å, 4.1 Å and 3.64 Å respectively⁴. The apparently small difference in the kinetic diameters of these two gases is thought to be one of the main reasons why zeolites and glassy polymers show preferential sorption and permeation of oxygen relative to nitrogen^{5,6}. We expect therefore that the repulsive interaction will increase the effective kinetic diameter of oxygen. Thus if air is diffused through a superconducting ceramic material at $T < T_c$, we expect preferential permeability of nitrogen, provided that the average pore diameter is low enough (~ 10 Å). We examine, as an example, the possibility of preferential permeability of nitrogen over that of oxygen

through $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ in the diamagnetic state. Such a separation process is expected to take place for a diffusion-controlled permeation of air through the superconducting pellet in the temperature range in which the diamagnetic susceptibility is fully developed. (The Meissner onset temperature in our samples was 102 K and the diamagnetic effect was fully developed at ~ 77 K.) The permeation experiment was therefore performed using a low-pressure gradient of 50 kPa starting at the lowest temperature possible, just above the dew point of air (81.3 K at 100 kPa (ref. 7)). Below the dew point, the results of the permeation experiments are obscured by the gas-liquid equilibria of oxygen and nitrogen.

We conducted an experiment (see Fig. 2) in which dry air was passed over and diffused through a $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ pellet, 13 mm in diameter and 1 mm thick, mounted in a brass block cooled by liquid nitrogen. The composition of the outflow gas ($\sim 0.4 \text{ ml min}^{-1}$ at 26°C) was analysed by a gas chromatograph in real time. The resulting run, measured at a heating rate of 0.1 K min^{-1} , in the temperature range between 82 K and 92 K, is shown in Fig. 3a. The critical transition temperature (T_c) for this pellet was $92 \pm 0.5 \text{ K}$, and the temperature dependence of the resistance is shown in Fig. 4a. This sample was quite porous, its specific density $\rho = 5.64 \text{ g cm}^{-3}$ being comparable with the specific theoretical density $\rho_{th} = 6.4 \text{ g cm}^{-3}$.

In a control experiment a $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet was prepared by a fast quench of a $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ pellet from 900°C to liquid-nitrogen temperatures. This sample did not exhibit the Meissner effect and a plot of resistance against temperature is shown in Fig. 4b. This pellet was similar in density and micro-grain structure to the superconducting sample used above. It is apparent from comparison of the plots in Fig. 3a and b that

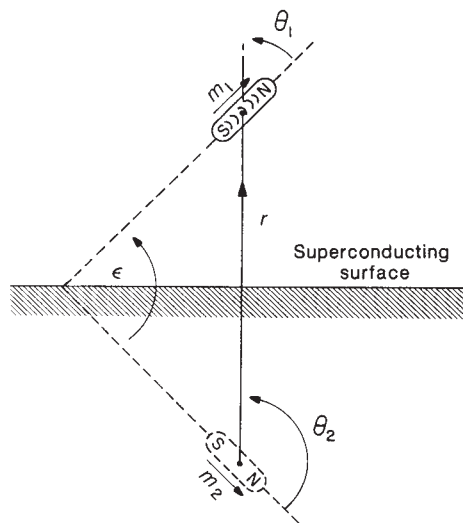


FIG. 1 The magnetic moment m_1 and its mirror image m_2 in the superconducting wall.

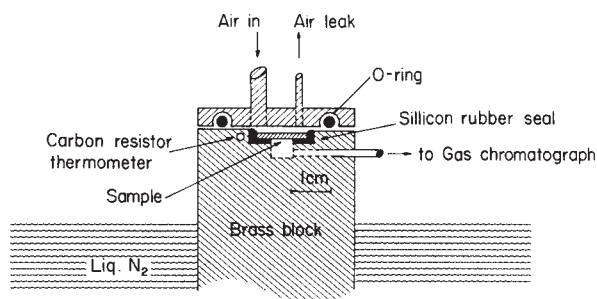


FIG. 2 Experimental set-up for measurement of O_2 and N_2 permeabilities through ceramic pellets at low temperature.

the superconducting sample does impede the mobility of O_2 for $T < 83 \text{ K}$, and indeed shows preferential permeability for nitrogen in comparison with that of the control. This trend is followed by a sharp desorption of the trapped oxygen for $T \approx 85 \text{ K}$; the permeate stream composition subsequently tails off to the O_2/N_2 ratio of 0.268, which is that observed in air.

We suggest that the experimental results may be interpreted as the manifestation of preferential permeability of nitrogen over that of oxygen in a temperature range in which the diamagnetism of the ceramic material is strong, followed by desorption of oxygen upon the increase of the magnetic susceptibility in the pellet.

In another experiment the relative permeation of O_2 against N_2 was measured through a 9-mm-thick $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ pellet and through a $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich sample of the same geometry using a pressure gradient of 80 kPa and a flow rate of $\sim 0.2 \text{ ml min}^{-1}$. The results, in the temperature range 85–88 K measured at a heating rate of 0.1 K min^{-1} , are presented in Table 1. In this experiment high selectivity is observed, with $(\text{O}_2/\text{N}_2)_{\text{air}}/(\text{O}_2/\text{N}_2)_{\text{permeate}} \approx 5$.

We expect that the application of a magnetic field, $H > H_{c1}$, to the diamagnetic membrane in the superconducting state will quench the selectivity for nitrogen against oxygen permeation that is observed for $T < T_c$ and $H = 0$. $\text{Bi}_{1.8}\text{Pb}_{0.2}\text{Ca}_2\text{Sr}_2\text{Cu}_3\text{O}_x$, a ceramic with a high T_c ($111 \pm 1 \text{ K}$) and low H_{c1} ($< 100 \text{ Oe}$ at 4.2 K), was prepared to test this prediction. The permeation experiment was conducted through a 2-mm-thick pellet using a pressure gradient of 50 kPa, a flow rate of 0.4 ml min^{-1} and a

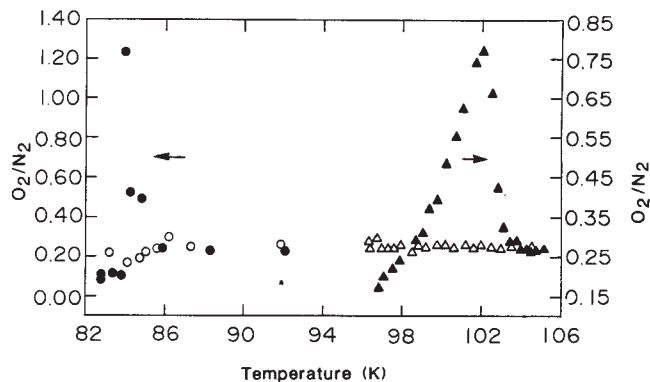


FIG. 3 O_2/N_2 ratio in the out-flow gas as a function of temperature, for $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ (●), a $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet (○), and $\text{Bi}_{1.8}\text{Pb}_{0.2}\text{Ca}_2\text{Sr}_2\text{Cu}_3\text{O}_x$ in a field of $H = 0$ (▲) and $H = 220 \text{ Oe}$ (△).

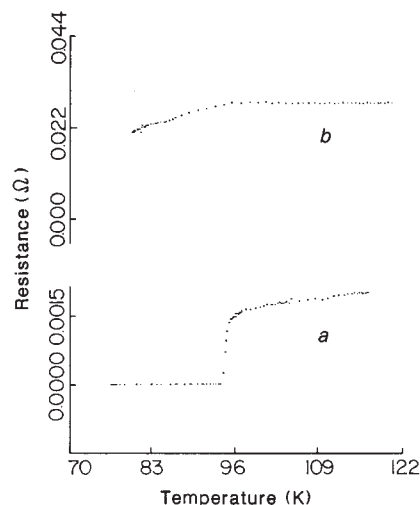


FIG. 4 Resistance against temperature for a, a $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ pellet, and b, a $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet.

heating rate of 0.1 K min^{-1} . Figure 3c, d shows the results for a magnetic field ($H=0$ and $H=220 \text{ Oe}$) applied perpendicularly to the surface of the pellet. The measured selectivity is shifted to a higher temperature range than that observed for $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ (see Fig. 3a, b) as is the desorption peak, which is at $\sim 102 \text{ K}$ for the zero-field run. A field of 220 Oe is large enough to quench the selectivity effect as the magnetic flux penetrates the diamagnetic membrane. (Note that at $\sim 100 \text{ K}$, $H_{c1} < 20 \text{ Oe}$.) $\square$

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Different behaviour of platinum in the Indian and Pacific Oceans

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THERE is little known about the chemical behaviour of the platinum-group metals in the ocean. Here we have used cathodic stripping voltammetry¹ to determine the concentration of dissolved platinum in the water column of the Indian Ocean. The range of Pt values obtained ($0.2\text{--}1.6 \text{ pM}$) is similar to that found previously in the Pacific Ocean²⁻⁴. But unlike the earlier Pacific Ocean investigations which depicted platinum as having nutrient-like behaviour, our data show that in the Indian Ocean the element is scavenged down the water column and so is depleted with depth. This is consistent with the predominant oxidation state of platinum in oxygenated sea water being Pt(IV) , which is also predicted from thermodynamic considerations and the association of platinum with manganese nodules in sediments². The data show that platinum co-varies in the water column with dissolved manganese, the concentration of Pt being lower than Mn by a factor of about 600. But the residence time of Pt in the oceans is longer than that of Mn, as revealed by a Pt/Mn ratio in the Indian Ocean that is 300 times higher than in crustal rock.

Seawater samples were obtained at $27^{\circ}00' \text{ S}$, $56^{\circ}58' \text{ E}$ (station CD 1504) and $06^{\circ}09' \text{ S}$, $50^{\circ}54' \text{ E}$ (station CD 1507) during cruise 15 of the RRS *Charles Darwin* in the Indian Ocean⁵. Clean laboratory and sampling procedures were used to avoid sample contamination⁶. The samples were collected using 10-litre polytetrafluoroethylene-lined Go-Flo bottles mounted on a rosette with conductivity, temperature and depth measuring instrumentation. The sea water was immediately filtered under pressure through acid-washed $0.4 \mu\text{m}$ 'Nucleopore' filters in teflon filter holders into acid-washed high-density polyethylene bottles and stored frozen. Before analysis, samples were thawed and acidified to pH 2 with HCl (Aristar grade), and a period of at least a week was allowed to elapse for desorption from the bottle walls and dissolution of any particulates formed in the process of freezing and thawing. The samples were then irradiated with ultraviolet light for 6–8 hours to break down

organic material such as surfactants and complexing ligands.

Dissolved platinum was determined by cathodic stripping voltammetry¹. When sulphuric acid (0.5 M), hydrazine (0.0015%) and formaldehyde (0.012%) were added to a 10 ml aliquot of each sample, the formazone produced formed a complex with Pt(II) . A small constant fraction of this complex is adsorbed on a hanging mercury drop electrode during a preconcentration stage that lasts for 5 min and catalyses the formation of hydrogen when the electrode potential is scanned in a negative direction. The reduction current associated with this hydrogen formation is linearly related to the combined Pt(II) and Pt(IV) concentration¹. Supplementary data such as the temperature, salinity, amounts of dissolved oxygen, nutrients and other metals were obtained during the cruise and are described elsewhere^{5,6}.

The vertical distribution of platinum, manganese and salinity at stations CD 1504 and CD 1507 is shown in Figs 1 and 2. Depletion with depth is shown in both Pt profiles and for Mn. The drop in the Pt concentrations in the upper water column occurs at a depth that is shallower at station CD 1507 than at station CD 1504. This is consistent with upwelling at station CD 1507 and with downwelling at station CD 1504, as evidenced by the shape of the nutrient/depth profiles.

Some co-variation of Pt and Mn is apparent from a linear regression of the Pt concentration (pM) as a function of the Mn concentration (nM) ($[\text{Pt}] = a[\text{Mn}] + b$), where $a = 1.06 \pm 0.28$ and $b = 0.30 \pm 0.31$. The standard deviations are quite large because of the variation in Mn concentrations in the deep waters (the Mn concentrations were almost at the limit of detection) and because of a scarcity of data for the upper water column, where most of the change in the platinum concentration occurs. Analysis of a number of samples without prior ultraviolet photolysis showed that interference by dissolved organic material, presumably consisting of surface-active as well as chelating compounds, was particularly strong in the upper water column. But this interference was removed in the ultraviolet-photolysis step, as was apparent from the voltammetric sensitivity (the ratio of the reduction current over the platinum concentration), which was constant for samples from all depths.

The ratio of crustal Mn to Pt is $\sim 2 \times 10^5$ (crustal data from Govett⁷), whereas the ratio of Mn to Pt in sea water is ~ 600 . Thus platinum is significantly enriched relative to manganese in sea water compared with its crustal value, which suggests that platinum is not as efficiently scavenged as manganese, and has a longer residence time. Calculation of the inorganic speciation of Pt(II) in sea water using published stability constants⁸ shows that it occurs largely (98%) as the PtCl_4^{2-} complex (which is an anion, and would tend to behave conservatively), the rest being made up of PtCl_5^- complexes. The overall α -coefficient⁹ of complexation of Pt(II) , $\alpha_{\text{Pt}} = [\text{Pt}_T]/[\text{Pt}^{2+}]$, where $[\text{Pt}_T]$ is

TABLE 1 The concentration of platinum in the Western Indian Ocean

Station CD 1504		Station CD 1507	
Depth (m)	Pt (pM)	Depth (m)	Pt (pM)
25	1.3	5	1.6
77	1.1	45	1.3
152	1.6	105	1.1
500	0.45	200	0.80
799	0.80	650	0.83
1,080	0.20	880	0.30
1,750	0.29	1,506	0.33
2,451	0.17	2,003	0.41
3,002	0.30	2,850	0.51
3,749	0.22	3,451	0.37
4,550	0.21	4,050	0.43
4,926	0.29	4,650	0.39
		4,855	0.36

The standard deviation of the Pt concentrations is $\pm 10\%$.

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the total Pt(II) concentration, is calculated from $\alpha_{\text{Pt}} = 1 + \Sigma K_{\text{PtCl}_i} [\text{Cl}^-]^i$ (hydrolysis of Pt^{2+} is negligible in the presence of Cl^-) and has a value of 10^{12} . The much greater value for α_{Pt} than for α_{Mn} (~ 2 ; ref. 10) may explain the enrichment of Pt in sea water relative to Mn, as uncomplexed cationic Mn(II) would tend to be scavenged more easily than the anionic chloride species of Pt(II).

The profiles for Pt in the Indian Ocean are quite different from those obtained in earlier studies from the Pacific, which showed that the element behaved like a nutrient²⁻⁴ (a comparative profile for Pt displaying this behaviour in the Pacific Ocean is illustrated in Fig. 2c). But because Pt is enriched relative to Pd in ferromanganese nodules, and because of the greater stability of Pt(IV) relative to Pd(IV), it has been proposed that Pt might also be subject to oxidative removal^{2,3}. The reduction of Pt(IV) to Pt(II) in an electrolyte containing chloride ions (sea water) is described by¹¹ $\text{PtCl}_6^{2-} + 2\text{e}^- \rightleftharpoons \text{PtCl}_4^{2-} + 2\text{Cl}^-$, and has a value of 0.68 V for E^0 . According to the Nernst equation, the ratio of Pt(II)/Pt(IV) in sea water depends on the potential, E ,

as $E = 0.67 - 0.03 \log ([\text{PtCl}_4^{2-}]/[\text{PtCl}_6^{2-}])$. The redox potential, E_{H} , of oxygenated water is determined by the $\text{O}_2/\text{H}_2\text{O}$ redox couple and lies at $E_{\text{H}} = 0.75$ V, whereas in surface sea water in the presence of 10^{-7} M hydrogen peroxide¹² it is controlled by the $\text{O}_2/\text{H}_2\text{O}_2$ couple and $E_{\text{H}} = 0.40$ V. The calculated equilibrium ratio of Pt(II)/Pt(IV) amounts to 10^9 in surface waters, whereas in deeper oxygenated waters it is 0.002. Therefore the thermodynamically preferred oxidation state of Pt is Pt(IV) in most of the oceanic water column, whereas in surface waters it is Pt(II). The scavenged behaviour of Pt in the Indian Ocean is consistent with this thermodynamic prediction. But perhaps it is not consistent with the rather long residence time of Pt which would be about 300 times longer than that of Mn on the basis of the Pt/Mn ratio in the crust as compared with the ratio in seawater.

The deep-water concentration of Pt in the Indian Ocean is lower than in the Pacific, whereas the concentration in the upper water column is greater, but the cause of this difference is not clear. Perhaps it results from small differences in the E_{H} arising from variations in the dissolved oxygen concentration or of

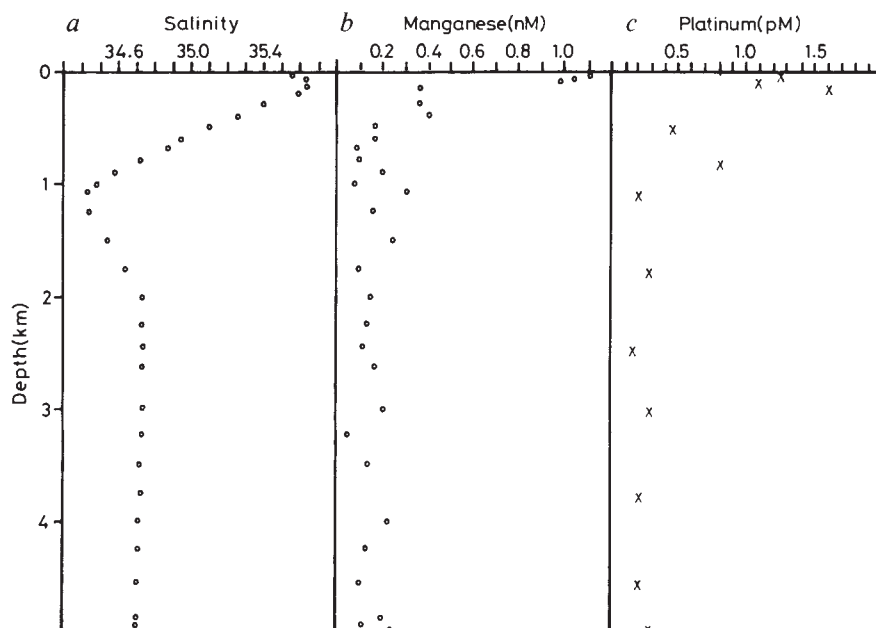


FIG. 1 Vertical distribution of *a*, salinity *b*, manganese (from N. H. Morley, P. Statham & J. D. Burton, manuscript in preparation) and *c*, platinum in Station CD 1504.

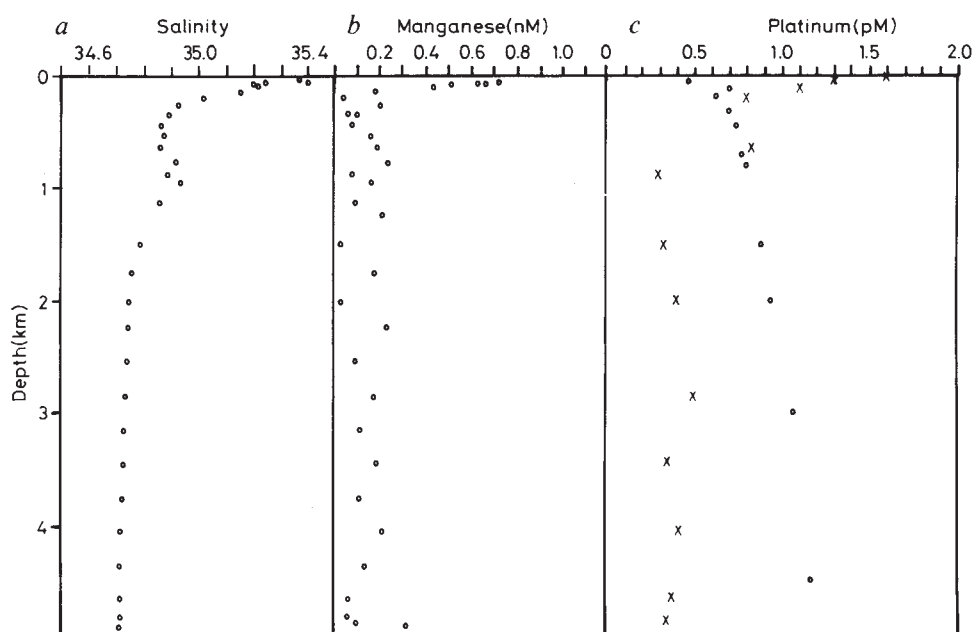


FIG. 2 Vertical distribution of *a*, salinity *b*, manganese (from N. H. Morley, P. Statham & J. D. Burton, manuscript in preparation) and *c*, platinum in Station CD 1507; in *c*, the platinum concentration in the Pacific Ocean³ (circles) is shown along with that in the Indian Ocean (crosses).

localized inputs from hydrothermal (in the Pacific deep waters) or eolian (surface Indian Ocean waters) origin. The possibility cannot be discounted that the difference is caused by an experimental artefact, perhaps as a result of organic trace metal speciation. For instance, it is possible that the radioactive tracer³ that was added to samples before Pt determination by GF-AAS, to verify the accumulation efficiency of the anion-exchange resin, did not reach equilibrium with organically complexed Pt. This is unlikely, as the samples were acidified to low pH; nevertheless, very stable metal-organic complexes such as of Cu and Ni occur in sea water^{13,14}, and a small amount of organically complexed copper is stable in acidified samples¹⁵. Furthermore, it is not impossible that complexation of Pt(II) by natural ligands is much stronger than with Cu(II) or Ni(II) if we consider the high value for α_{Pt} in sea water and the large shift in the reduction potential (>1 V, equivalent to a value for $\log \alpha_{\text{Pt-formazine}} > 30$) on complexation of Pt(II) by formazone before voltammetric analysis¹. Such organic complexing material, if present, should have been removed in the ultraviolet-photolysis step and would therefore not interfere with the voltammetric determination of Pt.

The interesting chemistry of platinum in the oceans should be investigated further if indeed the redox properties of Pt(IV)/Pt(II) and the strong complexation of Pt(II) can account for the observed differences in the Pt concentrations in the Pacific and Indian oceans. □

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Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties

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A FUNDAMENTAL step in visual pattern recognition is the establishment of relations between spatially separate features. Recently, we have shown that neurons in the cat visual cortex have oscillatory responses in the range 40–60 Hz (refs 1, 2) which occur in synchrony for cells in a functional column and are tightly correlated with a local oscillatory field potential. This led us to hypothesize that the synchronization of oscillatory responses of spatially distributed, feature selective cells might be a way to establish relations between features in different parts of the visual field^{2,3}. In support of this hypothesis, we demonstrate here that neurons in spatially

separate columns can synchronize their oscillatory responses. The synchronization has, on average, no phase difference, depends on the spatial separation and the orientation preference of the cells and is influenced by global stimulus properties.

We recorded multi-unit responses to appropriately oriented moving light bars simultaneously from 5 to 7 spatially separate sites in cortical area 17 of 13 adult cats. To determine the temporal relationship of the firing patterns recorded at two sites, we computed both the auto- and cross-correlation functions of the spike trains^{4,5}. For 132 of 199 recording sites, the auto-correlation function of the responses was periodic, indicating that the neuronal responses were oscillatory. To establish an objective criterion for the occurrence of oscillatory responses, we fitted a damped sine wave (Gabor function) to the auto-correlograms. Responses were considered to be oscillatory when the fitted function had at least three peaks and when the amplitude of the sinusoidal modulation was significantly different from zero ($P < 0.05$) and exceeded 10% of the amplitude of the cross-correlogram recomputed after shuffling the trial sequence by one stimulus period. The frequency of these oscillatory responses ranged from 40 to 60 Hz (mean, 50 ± 6 Hz), was similar for different recording sites in the same animal and depended only slightly on stimulus configuration (orientation, direction)². Of these 132 recordings, we selected 99 pairs in which oscillatory responses occurred simultaneously at two sites, and used these in cross-correlation analysis. Applying the same criteria used for auto-correlograms, 51 of the cross-correlograms had a significant correlation between the oscillatory responses.

Figure 1 illustrates a typical case in which neuronal responses were oscillatory and synchronized across spatially separate columns. Responses were recorded from five closely spaced sites near the representation of the area centralis of the retina. The receptive fields were overlapping but had different orientation preferences at adjacent sites. Stimulation with a light bar of 112° orientation evoked vigorous responses at sites 1, 3 and 5 but not at sites 2 and 4. As indicated by the periodic modulations of both auto- and cross-correlation functions, these responses were oscillatory and the oscillations were tightly correlated with zero phase difference. Changing the orientation of the stimulus to 22° , to maximize activation of the units at sites 2 and 4 produced synchronized oscillatory responses at these sites (data not shown). In all cases the correlations were abolished in the shuffled cross-correlogram^{4–11}.

When the recording sites had a larger spatial separation (>2 mm) the receptive fields were non-overlapping and could be stimulated independently. This enabled us to activate the

TABLE 1 Correlated oscillatory neuronal responses in area 17 as a function of spatial separation of recording sites and angular difference in preferred stimulus orientation

Angular difference of preferred orientation	Spatial separation	
	0.4–2.0 mm (overlapping fields)	2.0–7.0 mm (non-overlapping fields)
0–22°	90% (28/31)	54% (7/13)
45°	73% (8/11)	0% (0/8)
67–90°	44% (7/16)	25% (1/4)

Data were taken from a total of 99 cross-correlograms in which simultaneous oscillatory responses were recorded from two electrodes in area 17. Correlograms computed for responses at sites separated by 8–12 mm ($n=16$) were excluded. The correlograms were classified into six categories based on the difference in orientation preference of the neurons at the two recording sites and whether or not the receptive fields were overlapping. The results are presented as the percentage of recordings showing oscillatory correlations. The numbers in parentheses correspond to the number of oscillatory correlations and the total number of response pairs analysed for that category, respectively. The correlograms which showed no periodic modulation (48 out of 99) showed either a single peak centred around a 0 ms time delay ($n=11$) or a flat distribution ($n=37$).

units at each site even if their preferred orientations differed and to determine more precisely if the extent of correlations depended on the similarity of orientation preferences. Figure 2 illustrates the typical case where oscillatory responses in remote columns were synchronized if their orientation preferences were similar but showed no fixed phase relationship when the orientation preferences differed.

In two individuals we recorded at two sites separated by 7 mm in which the receptive fields were non-overlapping, had the same orientation preference and were aligned colinearly. This enabled us to co-activate the units at both recording sites with a single long light bar, as well as with two short, independently moving stimuli (Fig. 3). In both cases, the stimuli evoked oscillatory responses at each site. When the short light bars were moved in opposite directions over the two receptive fields, the respective responses showed no phase locking. When the two stimuli were moved in the same direction, however, the oscillations became weakly synchronized and this synchronization was markedly enhanced in each case when the responses were evoked with a single long light bar that co-stimulated the two receptive fields. This suggests that synchronization depends on global features of the stimuli such as coherent motion and continuity, which are not reflected by the local responses alone.

The probability for the occurrence of phase locking depended both on the distance between recording sites and on the angular difference between preferred stimulus orientations (Table 1). There was no phase locking of the oscillatory responses when the electrodes were separated by 7–12 mm ($n=16$). At intermediate distances of 2–7 mm, when the receptive fields of the recorded neurons were non-overlapping, phase locking occurred mainly between neuronal groups with similar orientation preferences. The same trend was observed for more closely spaced neurons (0.4–2.0 mm), which had overlapping receptive fields but in these cases phase locking was also observed for cells with different orientation preferences. Phase locking of oscillatory

responses typically occurred with a phase difference of 0 ms (32 out of 51) and the phase difference rarely exceeded ± 3 ms.

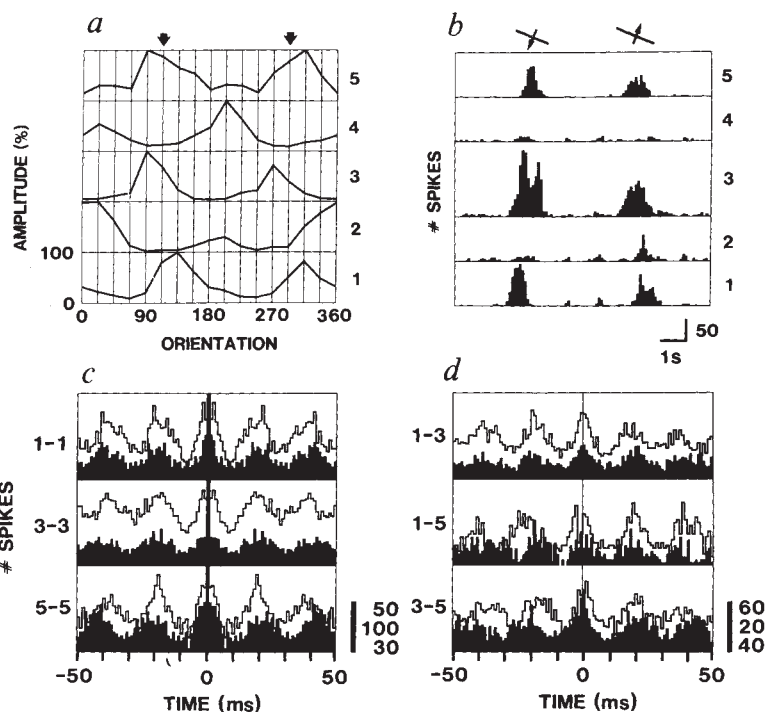
Our auto-correlation data confirm that the responses of a large fraction of cortical neurons are oscillatory and that these oscillations are synchronous for cells that are close enough together to be recorded with a single electrode^{1,2}. Thus, we could take advantage of multi-unit recordings for the cross-correlation analysis, considerably increasing the number of events per unit time and allowing us to confine the analysis to short epochs. Correlations between the firing probabilities of neurons in the visual cortex have been described previously^{6–12} and shown to be dependent on orientation preference^{6,9} but only one study has provided evidence for oscillatory correlograms¹¹. This relative lack of evidence for oscillatory responses in the cortex has several possible explanations. First, only a fraction of cortical neurons have oscillatory responses²; second, averaging procedures mask the oscillations because they are not phase-locked to the stimulus²; and third, previous studies may have excluded oscillatory activity because cross-correlograms of rhythmic responses were interpreted as misleading^{6,12}.

The system of tangential intracortical connections^{13–19}, or the reciprocal projections from other cortical areas²⁰ may provide the anatomical substrate for the synchronization of oscillatory responses between remote columns. Common input from sub-cortical structures can be excluded because collaterals of geniculate afferents do not span sufficiently large distances and do not have oscillatory responses in this frequency range^{1,2}.

We propose that the synchronization of oscillatory responses in spatially separate regions of the cortex may be used to establish a transient relationship between common but spatially distributed features of a pattern²¹. Our data show that synchronization is sensitive to global features of stimuli such as continuity, similarity of orientation and coherency of motion. Synchronization may therefore serve as a mechanism for the extraction and representation of global and coherent features

FIG. 1 Orientation-specific intercolumnar synchronization of oscillatory neuronal responses in area 17 of an adult cat. *a*, Normalized orientation tuning curves of the neuronal responses recorded from five electrodes spaced 400 μ m apart and centred on the representation of the area centralis. Response amplitudes (ordinate) to stimuli of different orientations (abscissa) are expressed as a percentage of the maximum response for each electrode. The arrows indicate the stimulus orientation (112°) at which the responses were recorded in *b*, *c* and *d*. *b*, Post-stimulus time histograms recorded simultaneously from the same five electrodes at an orientation of 112° . Note the small difference in the latencies of the responses indicating overlapping but slightly offset receptive field locations. *c*, Auto-correlograms of the responses recorded at sites 1 (1–1), 3 (3–3) and 5 (5–5). *d*, Cross-correlograms computed for the three possible combinations (1–3, 1–5, 3–5) between responses recorded on electrodes 1, 3 and 5. Correlograms computed for the first direction of stimulus movement are displayed with unfilled bars with the exception of comparison 1–5 in *d*.

METHODS. Adult cats were prepared for acute physiological recordings from the visual cortex using standard procedures². Anaesthesia was induced with a short acting anaesthetic (hexobarbital, 15 mg per kg or ketamine, 15 mg per kg) and then supplemented with a mixture of 30% O_2 , 70% N_2O and 0.1–0.3% halothane. Multi-unit activity was recorded from an array of 4–6 closely spaced (300–500 μ m) platinum-iridium electrodes (25 μ m tip diameter) and an additional single electrode that was moved independently. The array was inserted in the vicinity of the representation of the area centralis. The single electrode was positioned anteriorly and advanced down the medial bank of area 17. All receptive field locations were within 15° of the area centralis. Spikes exceeding a threshold of twice the noise level were detected with a window discriminator and digitized with a resolution of 1 ms. All recordings used binocular stimulation after the receptive fields for the two eyes had been aligned using prisms. When neurons recorded from different electrodes had overlapping receptive fields but differing orientation preferen-



ces, we used a stimulus orientation that evoked a response at each site of at least half the maximal amplitude. Responses that did not meet this criterion and that did not overlap in time were excluded from the analysis. In the case of non-overlapping receptive fields we applied two independently controllable stimuli. For each trial the stimuli were moved across the receptive fields, at the preferred velocity, in both directions of movement perpendicular to the axis of orientation. Each trial lasted for 10 s and was repeated 10 times.

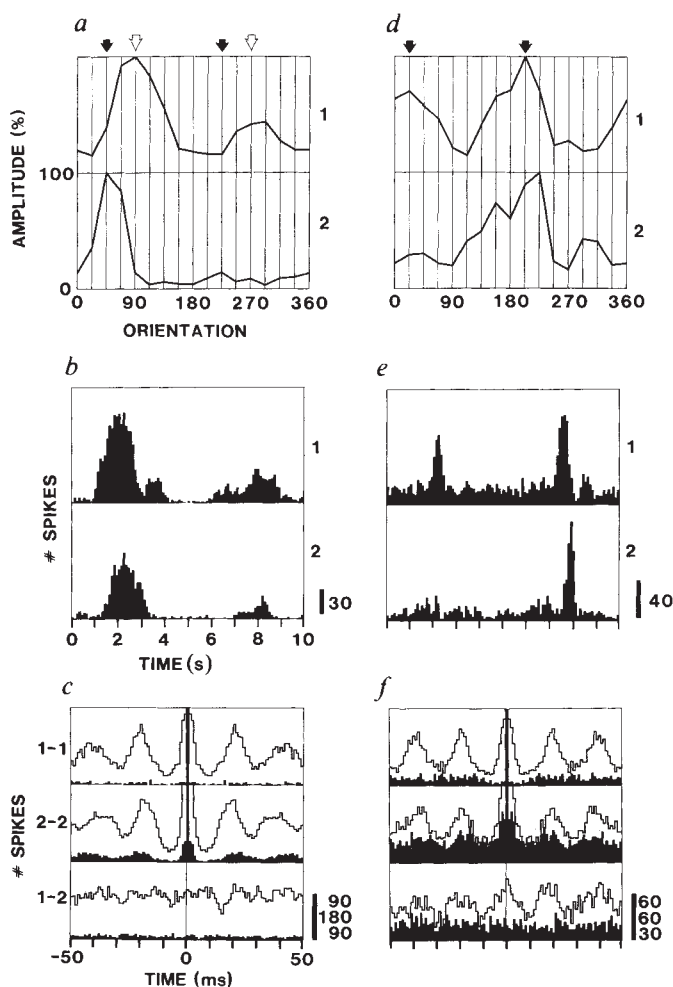


FIG. 2 Long-range inter-columnar synchronization of oscillatory responses depends on the similarity of orientation preference. *a*, Orientation tuning curves of multi-unit responses recorded at two sites (1 and 2) separated by 6 mm in area 17. The neurons had non-overlapping receptive fields and a clear difference in orientation preference of 45° (open and closed arrows). *b*, Post-stimulus time histograms of the responses recorded to stimulation of each receptive field at its own respective optimal orientation (open and closed arrows in *a*). *c*, Auto- (1-1, 2-2) and cross-correlograms (1-2) computed from the neuronal responses recorded from both electrodes revealed that oscillatory responses occurred at both sites but were not correlated. Unfilled bars are for the first direction of stimulus movement. *d*, Orientation tuning curves of multi-unit responses recorded from the same animal at two different cortical sites (1 and 2), separated by 7 mm. The neuronal responses at each site had similar orientation and directional preferences. *e*, Post-stimulus time-histograms of the activity recorded at each site in response to their optimal stimulus orientation of 22° (arrows in *d*). *f*, Auto- (1-1, 2-2) and cross-correlograms (1-2) computed from the neuronal responses recorded on the two electrodes demonstrate oscillatory responses at both sites which were correlated with zero phase difference. Unfilled bars are for the second direction of stimulus movement.

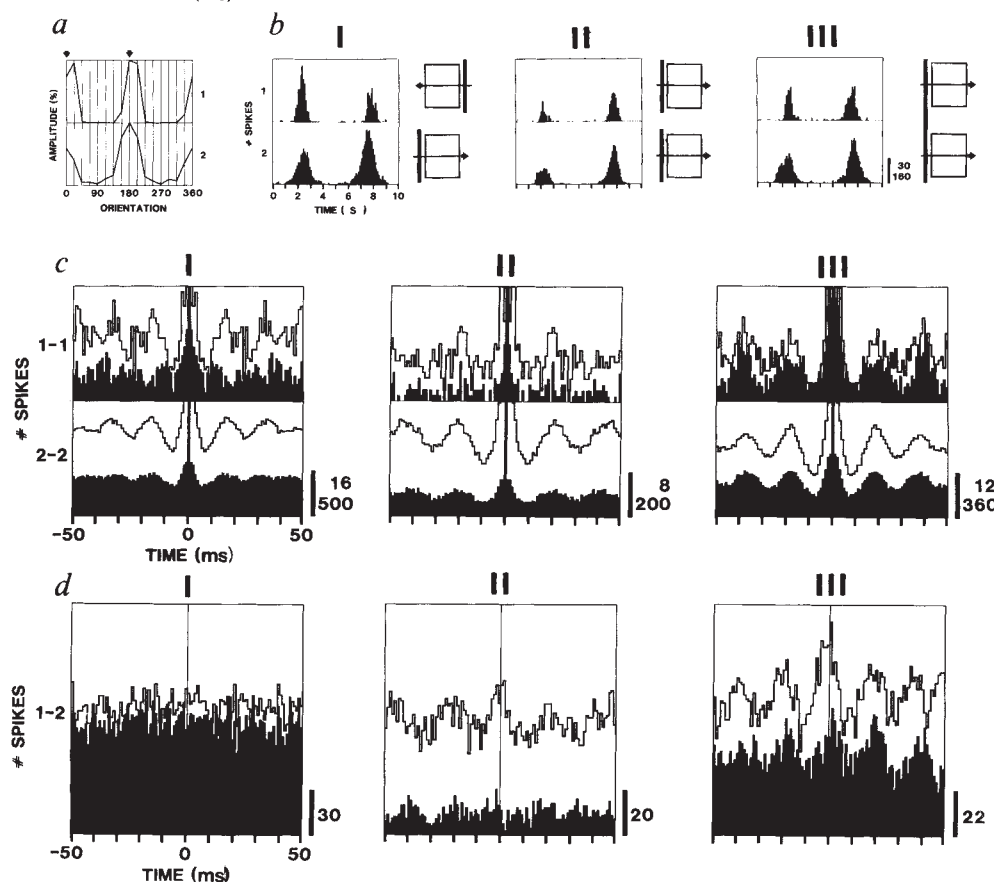


FIG. 3 Long-range oscillatory correlations reflect global stimulus properties. *a*, Orientation tuning curves of neuronal responses recorded from two electrodes (1, 2) separated by 7 mm show a preference for vertical light bars (0 and 180°) at both recording sites. *b*, Post-stimulus time-histograms of the neuronal responses recorded at each site for each of three different stimulus conditions: (I) two light bars moved in opposite directions; (II) two light bars moved in the same direction; and (III) one long light bar moved across both receptive fields. A schematic diagram of the receptive field locations and the stimulus configuration used is displayed to the right of each post-stimulus time histogram. *c*, *d*, Auto-correlograms (*c*, 1-1, 2-2) and cross-correlograms (*d*, 1-2) computed for the neuronal responses at both sites (1 and 2 in *a* and *b*) for each of the three stimulus conditions (I, II, III) displayed in *b*. For each pair of correlograms except the two displayed in *c* (I, 1-1) and *d* (I) the second direction of stimulus movement is shown with unfilled bars.

of a pattern. Such processes are crucial for the analysis of visual scenes and figure-ground segregation^{3,22-26}. Synchronization of oscillatory responses may however also have a more general function in cortical processing because it is a powerful mechanism for establishing cell assemblies that are characterized by the phase and the frequency of their coherent oscillations. □

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Transcripts of one of two *Drosophila* cyclin genes become localized in pole cells during embryogenesis

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CYCLINS, originally discovered in the eggs of marine invertebrates, are proteins which undergo dramatic cycles of synthesis followed by degradation at the metaphase-anaphase transition of cell division¹⁻³. That they participate in the G₂-M transition is supported by the fact that when synthetic cyclin messenger RNAs from clam and sea urchin are microinjected into the G₂-arrested oocytes of *Xenopus*, they induce maturation^{2,4}. The cyclin of fission yeast is the product of the *cdc13* gene, which is known to interact with *cdc2*, a gene required for the entry into mitosis⁵⁻¹⁰. We have cloned the genes that encode A-type and B-type cyclins from *Drosophila melanogaster* by virtue of their sequence similarity to oligonucleotides corresponding to conserved regions of the cyclin genes. We show that both genes encode abundant maternal mRNAs, but whereas the cyclin A mRNA is relatively uniformly distributed before cell formation, the cyclin B mRNA becomes localized to the developing pole cells. In larvae, cyclin A is expressed predominantly in brain and imaginal disks, whereas cyclin B transcripts are abundant in testes.

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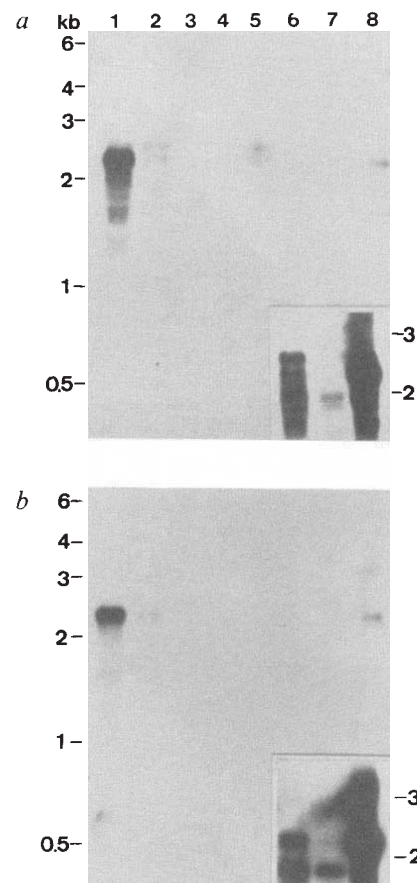


FIG. 1 Developmental northern blots showing the transcripts detected by cDNA clones of cyclins A (a) or B (b). Poly (A⁺) RNA from the following developmental stages was analysed: lane 1, 0-2 h embryos; lane 2, 2-4 h embryos; lane 3, first instar larvae; lane 4, second instar larvae; lane 5, third instar larvae; lane 6, pupae; lane 7, adult males; lane 8, adult females. The autoradiograph shown was exposed for 15 h. The inset in the lower right-hand corner of each autoradiograph is an 80-h (cyclin A) or 60-h (cyclin B) exposure in which only the region containing RNA of ~2-kb from lanes f-h is displayed. Filters were reprobbed with the *Drosophila ras* gene (*Dmras64B*) (ref. 16) as a control for uniform loading of RNA. The autoradiograph exposures indicated that the cyclin transcripts are 1-2 orders of magnitude more abundant than *ras* RNA in *Drosophila* embryos.

METHODS. An adult female *Drosophila* cDNA library in the bacteriophage vector λ gt10 was screened with a ³²P-labelled oligonucleotide mixture of sequence: AA(A/G)TA(T/C)GA(A/G)GA(A/G)ATTA(T/C)CC (probe 1). Hybridizations and washings were carried out as recommended by Wood *et al.*¹⁷. Duplicate filters were screened in parallel with a second oligonucleotide mixture: AT(T/C/A)(C/T)T(G/A)TGA(T/C)-TGG(T/C)TGT (probe 2). Positive plaques were purified by rescreening following the same protocol. The cDNAs fell into two classes: those that hybridized to both probes, and those that hybridized only to probe 1, cyclins A and B, respectively. We isolated three cyclin A cDNAs and four cyclin B cDNAs from ~5 × 10⁴ recombinant phage in this library. On rescreening, a 0-2 h embryo cDNA library, we isolated both cyclin A and B clones at a frequency of 0.002. The amino-acid sequence corresponding to probe 1 can be recognized within the amino-acid sequence derived from the sequence of the cDNA clones and is shown below in relation to amino acids 249-261 of clam cyclin A², and the corresponding regions of sea urchin cyclin⁴ and *cdc13* (refs 7 and 8):

Clam A	L	A	A	K	Y	E	E	I	Y	P	P	D	V
Sea urchin	I	A	S	K	Y	E	E	M	Y	P	P	E	I
<i>cdc13</i>	I	A	S	K	Y	E	E	V	M	C	P	S	V
<i>Drosophila</i> A	I	A	A	K	Y	E	E	I	Y	P	P	E	V
<i>Drosophila</i> B	I	A	T	K	Y	E	E	L	F	P	P	A	I

The amino-acid sequence corresponding to probe 2 (in relation to amino-acids 196-208 of clam cyclin A) is as follows:

Clam A	M	R	C	I	L	V	D	W	L	V	E	V	S
Sea urchin	M	R	L	I	L	V	D	W	L	V	Q	V	H
<i>cdc13</i>	M	R	G	I	L	T	D	W	L	I	E	V	H
<i>Drosophila</i> A	M	R	S	I	L	I	D	W	L	V	E	V	S
<i>Drosophila</i> B	M	R	A	V	L	I	D	W	I	N	E	V	H

The cyclin A cDNAs hybridize *in situ* to salivary gland chromosomes at 68E, and thus correspond to a gene that has been independently cloned by Lehner and O'Farrell¹⁸ and Y.-N. Jan (personal communication). The cyclin B gene hybridizes *in situ* to chromosome 2 at 59A. Northern blots using the cloned cDNAs as probes have been described previously¹⁹.

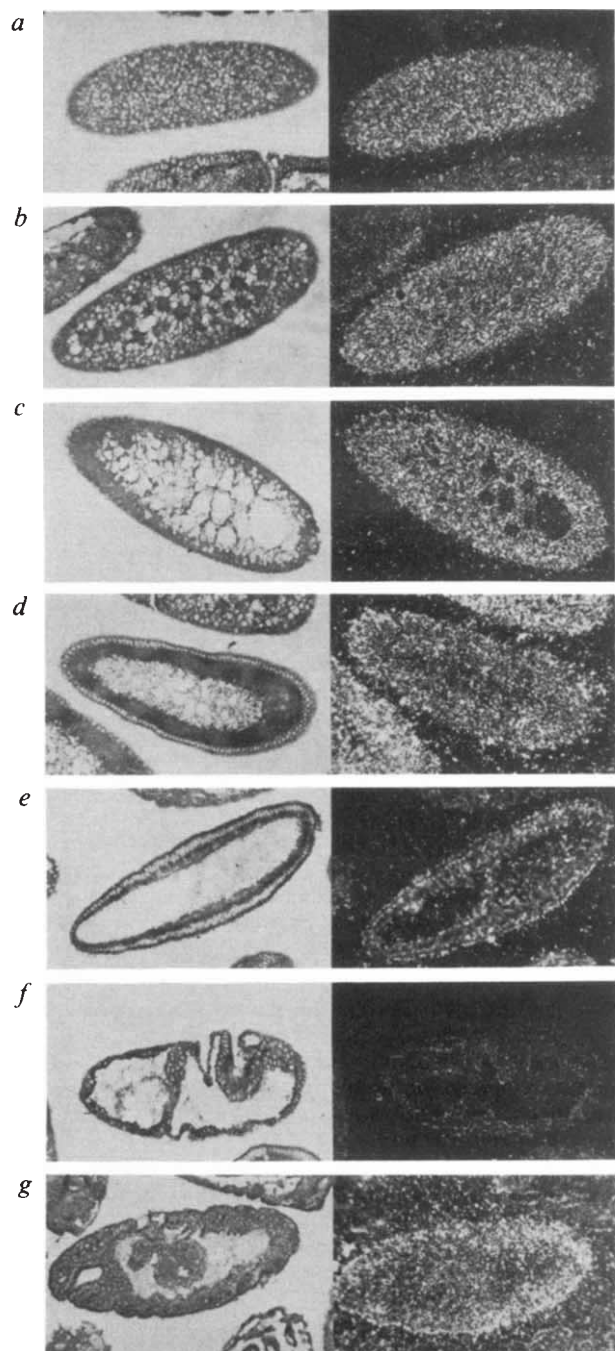


FIG. 2 *In situ* hybridization of a cyclin A cDNA probe to sections of embryos at various developmental stages. Bright-field and dark-field images are shown in the left and right panels respectively and embryos are oriented with the anterior pole to the left. Stages are according to Campos-Ortega and Hartenstein²⁰. *a*, Early stage 2 embryo showing uniform distribution of signal. *b*, Late stage 2 embryo (nuclear cycle 7–8) showing uniform grain distribution. *c*, Early stage 4 embryo (nuclear cycle 10) in which the signal is becoming distributed toward the cortex. *d*, Late stage 4 embryo (division cycle 12). *e*, New cellular embryo (stage 6) with weaker signal throughout cellular region. *f*, Stage 8 embryo, mid-way through germ-band elongation showing extremely weak signal. *g*, Stage 11 embryo with fully elongated germ-band showing signal corresponding to zygotic transcripts. Hybridizations *in situ* were carried out according to refs 21 and 22, except that after labelling in a random oligonucleotide-priming reaction (20 μ l), the reactions were stopped by the addition of 1 μ l 0.5 M EDTA and 20 μ l 20 mM DTT. HCl (2 μ l, 5 M) was added and the incubation continued at 37 °C for 10 min, followed by the addition of NaOH (4 μ l, 5 M) and a further 20-min incubation. The reactions were neutralized with HCl (2 μ l, 5 M) and Tris-HCl (2 μ l, 2 M, pH 7.5).

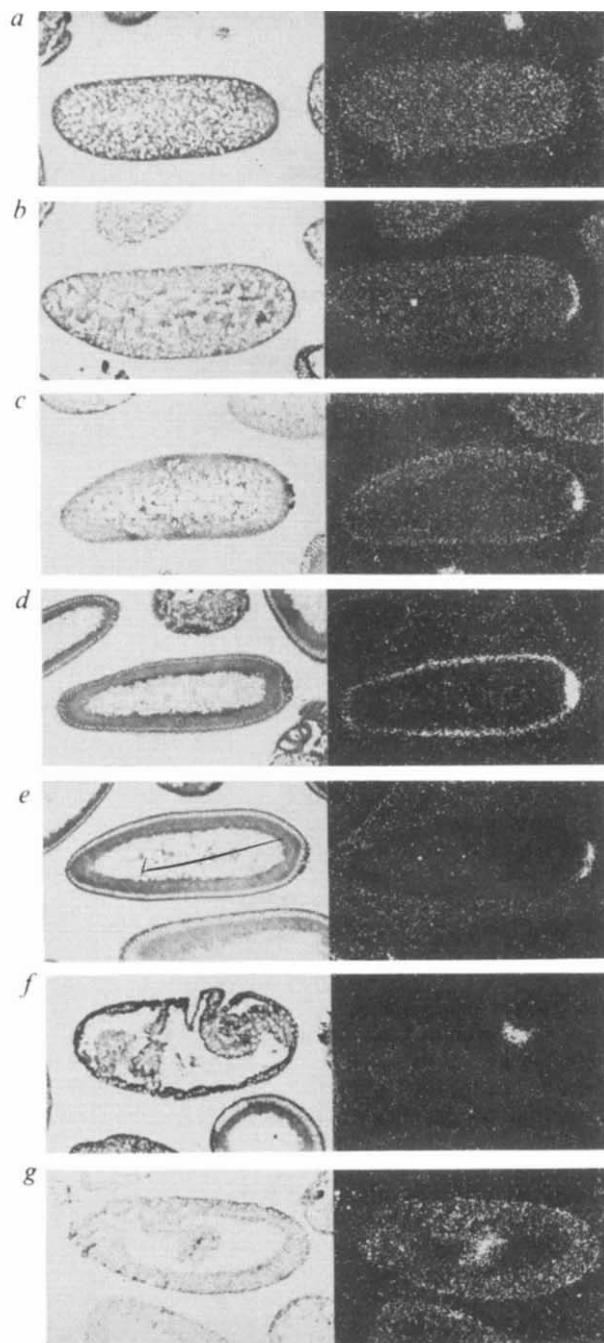


FIG. 3 *In situ* hybridization of a cyclin B cDNA probe to sections of embryos at various developmental stages. Bright-field and dark-field images are shown in the left and right panels, respectively. *a*, Early stage 2 embryo showing uniform distribution of signal. *b*, Late stage 2 embryo (nuclear cycle 8) showing transcripts beginning to concentrate at the posterior pole. *c*, Late stage 3 embryo (nuclear cycle 9) in which pole cells have just formed and are prominently labelled. Remaining labelling is becoming distributed towards the cortex. *d*, Late stage 4 embryo (division cycle 12) with all the signal tightly distributed in the vicinity of the nuclei at the cortex. Pole-cell labelling is conspicuous. *e*, An embryo forming cells (stage 6) in which somatic signal has disappeared leaving grains only over pole cells. *f*, Stage 8 embryo, mid-way through germ-band elongation showing labelling exclusively over pole cells. *g*, Stage 11 embryo with fully elongated germ-band showing signal prominent pole cell labelling and onset of signal corresponding to zygotic transcripts.

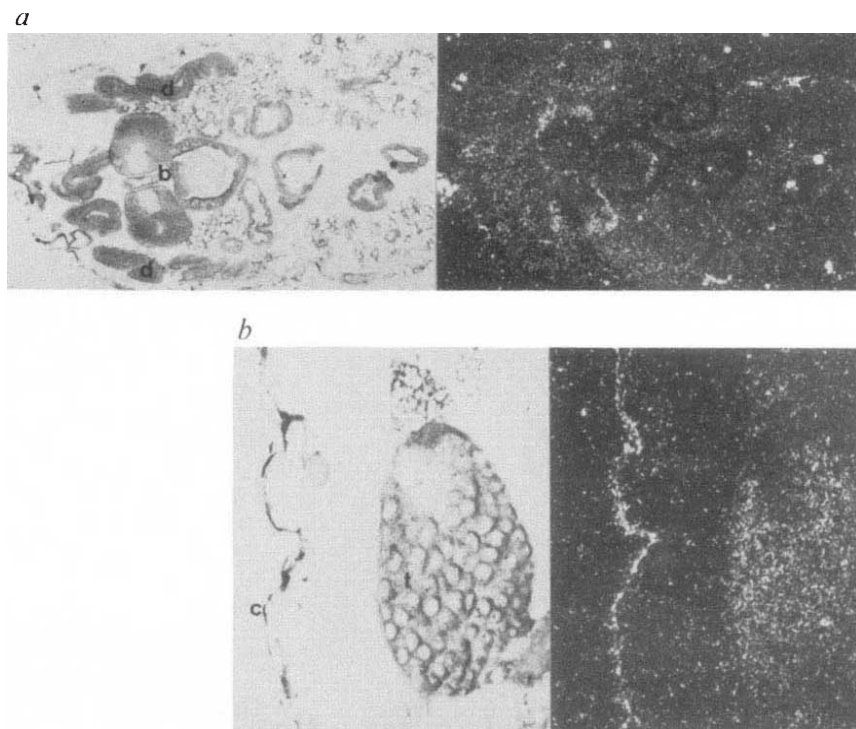


FIG. 4 *a*, *In situ* hybridization of a cyclin A cDNA probe to a section of a third instar larva. The anterior of the larva is facing to the left, and bright-field and dark-field images are shown in the left and right panels, respectively. The strongest labelling is seen in distinct regions of the three lobes of the brain (*b*). The imaginal disks (*d*) show a much lower level of more uniform labelling just above background. *b*, *In situ* hybridization of a cyclin B probe to a section of a third instar larva. This part of the section shows labelling of the testes (*t*) close to the larval cuticle (*c*). The signal in the cuticle is likely to be non-specific as it has been observed with many other probes (E.S., unpublished observations; M. Akam, personal communication). Fixation and embedding of larvae was carried out according to ref. 23.

We have cloned complementary DNAs corresponding to transcripts encoding proteins which, on the basis of sequence comparisons with the cyclins of other organisms, we refer to as cyclins A and B (see Fig. 1 legend). Both these cDNAs detect 2.3-kilobase (kb) transcripts that are most abundant in pre-cellular embryos, and also occur at high levels in adult females, as would be expected for maternally provided mRNA (Fig. 1). A 2.5-kb zygotic transcript of the cyclin A gene appears after cell formation (Fig. 1*a*, lane *b*), and is present at low levels throughout subsequent development. Three cyclin A transcripts are detectable in pupae, only one of which is present in adult males at low levels (Fig. 1*a*, inset). The developmental distribution of cyclin B transcripts is similar, except that a zygotic transcript of a different size is not seen, and a male-specific transcript of 2 kb is readily detectable (Fig. 1*b* inset, lane *g*).

The abundance of these transcripts in early embryos is consistent with the expected role of cyclins in the 13 cycles of mitosis that occur in the first 2 hours of development^{11,12}. We have examined the distribution of transcripts during embryogenesis by *in situ* hybridization and find that maternal message is initially distributed uniformly throughout the embryo (Fig. 2*a, b*). There seems to be a broad redistribution of the cyclin A transcripts towards the cortex during cycles 8 and 9 which continues in subsequent cycles. This could be either an active process in which mRNA migrates with mitotic apparatus and nuclei, or a more passive process by which transcripts are excluded from the interior region of the embryo occupied by the coalescing yolk (Fig. 2*c–e*), although other explanations, including the differential degradation of RNA, are possible. The abundance of the cyclin A transcripts falls dramatically following cell formation (Fig. 2*f*). At later stages, the cell cycle lengthens to 1–2 hours and two or three additional rounds of division occur in spatially restricted domains of the embryo (ref. 13, and V. Foe, personal communication). During these stages, zygotic transcripts appear (Fig. 2*g*) which, we surmise, correspond to the 2.5-kb RNA seen in the northern blots (Fig. 1).

Maternal cyclin B transcripts are first found uniformly throughout the embryo (Fig. 3*a*). They then undergo a dramatic redistribution such that by cycles 8–9, when nuclei are migrating to the cortex, cyclin B transcripts are becoming concentrated at the posterior pole (Fig. 3*b*). It is also possible that this represents *de novo* transcription in the nuclei reaching the posterior pole.

These nuclei undergo cellularization several cycles before the majority to form pole cells (Fig. 3*c*). As the somatic nuclei complete their syncytial mitotic cycles, cyclin B transcripts show a clear association with the cortex near nuclei (Fig. 3*d*). These cortical transcripts disappear abruptly during cell formation of somatic nuclei (Fig. 3*e*). Transcripts either persist or continue to be produced in the pole cells as they move dorsally and anteriorly in germ-band elongation (Fig. 3*f*). Some zygotic cyclin B transcription seems to ensue in the soma in later embryos, although the predominant labelling is still detected in pole cells (Fig. 3*g*). The redistribution of cyclin B mRNA in the syncytial embryo suggests that these transcripts contain localization signals that allow them to remain close to dividing somatic nuclei and the developing pole cells. Other maternal mRNAs are known to have specific distributions in early embryos: the graded distribution of *bicoid* mRNA, with its maximum concentration at the anterior pole of the *Drosophila* embryo, for example, requires a specific sequence present at its 3' untranslated end¹⁴.

After the hatching of the embryo, most of ensuing larval development involves cell growth and polytenization in the absence of cell division. The exceptions are the imaginal tissues that must proliferate to form adult structures, neuroblasts within the larval brain, and the mitotic lineages of the germ cells. We find that expression of cyclin A, but not cyclin B, is readily detectable in the periphery of the three brain lobes, and, at a lower level, in imaginal disks of third instar larvae (Fig. 4*a*). Conversely, cyclin B transcripts predominate in the larval testes (Fig. 4*b*), and presumably correspond to the prominent male-specific 2-kb cyclin B RNA, detected by northern blotting (Fig. 1).

Several aspects of germ-cell proliferation are significantly different from mitotic cell division in somatic tissues. In the imaginal discs, for example, there is a target cell number that may be controlled by cell-cell contacts of a certain pattern¹⁵. By contrast, cell division continues in the stem cells of the testes and ovary throughout the life of the organism to produce daughter stem cells and primary gonial cells. In addition, the premeiotic mitoses of the gonial cells in both male and female are accompanied by incomplete cytokinesis to produce groups of cells that share cytoplasm connected by canals. In spermatogenesis, the gonial cells undergo 4 mitotic and 2 meiotic

divisions within cysts to produce 64 spermatids. In oogenesis a cyst of 16 interconnected cells develops, of which 14 will undergo polyploidization to form the nurse cells and one will become the oocyte, *per se*, and undergo meiosis. One might speculate that there is a need for a specific cyclin either to maintain stem-cell proliferation (to provide for the peculiarities of what are essentially syncytial divisions in the gonial cysts) or to mediate the ultimate transition between mitotic and meiotic divisions. □

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A diacylglycerol analogue reduces neuronal calcium currents independently of protein kinase C activation

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DIACYLGLYCEROL analogues (for example 1,2-oleoylacetyl-glycerol, OAG) and phorbol esters are activators of protein kinase C, and have been widely used to study the function of this enzyme in both intact cells and cell-free preparations^{1,2}. Electrophysiological studies have shown that these activators can either depress^{3–6} or increase Ca²⁺ currents^{7–9}, or decrease K⁺ currents^{10,11} when applied outside the cell. It has been assumed that these effects are mediated by protein kinase C activation. Here we report that micromolar levels of OAG and phorbol esters depress Ca²⁺ currents in chick sensory neurons independently of their effect as activators of protein kinase C. The depression of the Ca²⁺ current is rapid and is unaffected by intracellular application of the protein kinase C inhibitors staurosporin, sphingosine and H-7¹². Furthermore, the activators were ineffective when applied intracellularly, indicating that their site of action is on the outside of the membrane.

Intracellular patch-clamp recordings were performed in acutely isolated dorsal root ganglion cells from 10–13-day-old

chick embryos^{13,14}. Figure 1a shows the change in action potential waveform induced by OAG¹⁵ (5 µM), which was delivered by pressure application through a micropipette positioned 10–20 µm from the cell. OAG reversibly depressed the amplitude of the spike and slightly prolonged its duration.

Under voltage-clamp, OAG reduced the total membrane current (see Fig. 1b), but isolated voltage-dependent Na⁺ currents, and also K⁺ currents³, were unaffected. A large, reversible decrease in the amplitude of isolated Ca²⁺ currents was recorded (Fig. 1c; see also ref. 3). A subsequent reduction of a Ca²⁺-activated K⁺ conductance could account for the decrease in the outward current shown in Fig. 1b.

Ca²⁺ currents recorded under the conditions of Fig. 1c typi-

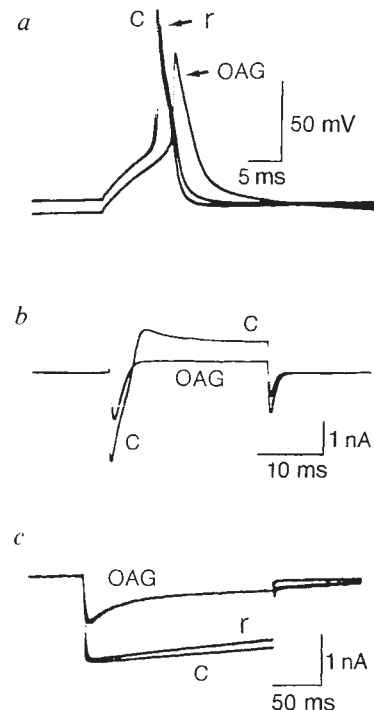


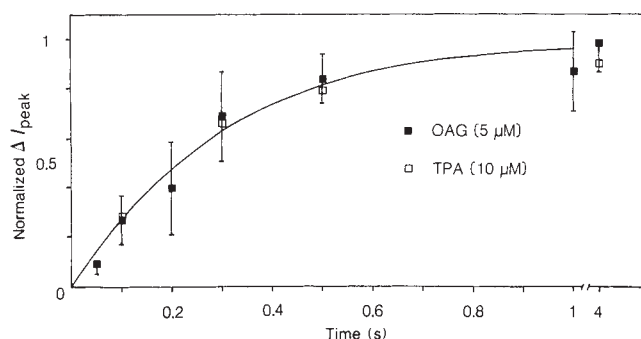
FIG. 1. Whole-cell recordings from chick dorsal root ganglion neurons before (c, control), during and after (r, recovery) 5-s exposures to 5 µM OAG. a, OAG application reduced the action potential amplitude and delayed repolarization by several ms (resting potential –55 mV). Recovery was 13 s after OAG application. Small hyperpolarizations (5–10 mV) were due to a non specific effect of the pressure application and were also observed when external saline was applied. b, Under voltage-clamp, both inward and outward currents were reversibly depressed by OAG (same cell as in a). c, OAG application reversibly reduced Ca²⁺ currents. Activating steps were from –50 (b) and –80 mV (c) to 0 mV.

METHODS. Electrode resistances were 3–5 MΩ. Normal external salines contained (in mM): NaCl, 130; KCl, 5.4; MgCl₂, 2; CaCl₂, 2; HEPES 10 (pH 7.3). The internal electrode solution contained (in mM) potassium aspartate, 130; EGTA 10; CaCl₂, 1; Mg-ATP, 2; HEPES, 10 (pH 7.3). Ca²⁺ currents were recorded in isolation in external saline containing (in mM): choline chloride, 120; CaCl₂, 5; MgCl₂, 2; HEPES, 10 (pH 7.3) and internal solution containing (in mM) CsCl, 100; TEA-Cl, 20; Mg-ATP, 2; EGTA, 10; CaCl₂, 0.1; HEPES, 40 (pH 7.3). Sodium currents were analysed using external saline containing (in mM) NaCl, 120; NiCl₂, 5; MgCl₂, 2; HEPES, 10 (pH 7.3). Potassium currents were analysed using external saline containing (in mM) choline chloride, 150; NiCl₂, 5; MgCl₂, 2; HEPES, 10 (pH 7.3) and internal electrode solution containing (in mM) potassium aspartate, 130; MgCl₂, 2; EGTA, 10; HEPES, 10 (pH 7.3). The temperature was 22 °C. Stock solutions of OAG (5 mM, Sigma) were prepared in chloroform and diluted in external saline. OAG did not immediately dissolve in the chloroform, as solutions tested the same day had no effect. Stock solutions were then stored for 3–4 weeks at 4 °C after which they did depress Ca²⁺ currents. Stock solutions of diC₈ (50 mM, Sigma), sphingosine (200 mM, Sigma) and H-7 (50 mM, Sigma) were prepared in chloroform and stock solutions of staurosporin (1 mM, Boehringer) were prepared in dimethylsulphoxide. Phorbol esters (1–11 mM, Sigma) were dissolved in ethanol. Stock solutions were stored at 4 °C. Sonification improved the solubility of the compounds.

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FIG. 2 Rate of reduction of the Ca^{2+} current during application of 5 μM OAG ($n=5$) and 10 μM TPA ($n=2$). The measurements were made using a delivery system that exchanges the solution surrounding the cell in about 50 ms (ref. 22). The difference between peak currents recorded after prolonged washout and during drug applications of various duration was normalized to the difference between peak currents recorded after washout and after prolonged (20 s) drug application.



cally inactivated with a time constant of several hundred ms, as described for high-threshold (L-type) Ca^{2+} currents¹⁶⁻¹⁹. During OAG application, the reduced Ca^{2+} current inactivated faster when 0.1% chloroform was used as a solvent but not with lower concentrations. Recovery was slower than that of the peak current. Similar results were obtained with another diacylglycerol analogue, *sn*-1,2-dioctanoylglycerol (diC_8)²⁰, and the three phorbol esters, *O*-tetradecanoylphorbol-13-acetate (TPA, phorbol myristyl acetate), phorbol-12,13-diacetate (PDA) and 4- α -phorbol-12,13-didecanoate (4- α -PDD).

We did not record shifts in the current-voltage relationship or changes in the cell leakage conductance during application of these agents (see also refs 3-5). Steps from -80 mV to 0 mV activated both high and low threshold (T-type) Ca^{2+} currents¹⁶⁻¹⁹. We found that the T-type current, which constitutes about 15% of the total Ca^{2+} current, was also transiently and reversibly blocked by OAG; similar observations have been made in GH3 cells⁵.

Table 1 (top) summarizes our results with diacylglycerol analogues and phorbol esters. Phorbol esters and diC_8 were less effective than OAG even at 10-fold higher concentrations. Control solutions of either glycerol or the solvents had no significant effect on the peak Ca^{2+} current. Interestingly, 4- α -PDD, which does not activate protein kinase C²¹, was as effective as the other phorbol esters at depressing Ca^{2+} currents.

TABLE 1 Effects of activators and inhibitors of protein kinase C

External pipette	Internal pipette	Per cent decrease I_{peak} (mean $\pm$ s.d.)	n
0.5 μM OAG	—	2 $\pm$ 2	7
5 μM OAG	—	58 $\pm$ 24	18
5 μM diC_8	—	2 $\pm$ 1	3
50 μM diC_8	—	18 $\pm$ 9	6
50 μM glycerol	—	0 $\pm$ 2	3
10 μM TPA	—	18 $\pm$ 5	6
10 μM PDA	—	15 $\pm$ 5	6
10 μM 4- α -PDD	—	17 $\pm$ 3	3
0.1% chloroform	—	3 $\pm$ 1	4
0.1% ethanol	—	3 $\pm$ 2	4
5 μM OAG	1 μM staurosporin	51 $\pm$ 17	6
5 μM OAG	50 μM sphingosine	75 $\pm$ 15	6
5 μM OAG	200 μM sphingosine	89 $\pm$ 9	5
5 μM OAG	50 μM H-7	72 $\pm$ 12	5
10 μM TPA	50 μM H-7	12 $\pm$ 6	7
10 μM PDA	50 μM H-7	8 $\pm$ 5	5
50 μM diC_8	50 μM H-7	30 $\pm$ 10	6
1 μM staurosporin	—	2 $\pm$ 1	3
50 μM sphingosine	—	5 $\pm$ 4	3
200 μM sphingosine	—	32 $\pm$ 8	3
50 μM H-7	—	31 $\pm$ 10	5

All agents were delivered through micropipettes for either 5 s externally or for a minimum of 10 min internally. Chloroform was the solvent for OAG and diC_8 , and ethanol was used to dissolve the phorbol esters (see text for details). I_{peak} , peak voltage-dependent Ca^{2+} current; n , number of cells examined.

We measured the onset of Ca^{2+} current reduction by OAG and TPA using a rapid delivery system²² (Fig. 2). Inhibition developed in less than 50 ms and was half-maximal by 200 ms. The offset of the response was similarly fast, indicating that the action was probably limited by diffusion. These results indicate that the drugs may be acting on the outside of the membrane, and not through an enzymatic pathway on the inner membrane.

To examine this possibility more directly, we tested whether the activators could depress Ca^{2+} currents after inhibition of protein kinase C. Table 1 (middle) summarizes our results from cells perfused internally with either staurosporin²³ sphingosine^{24,25} or H-7²⁶. We used H-7 and staurosporin at concentrations that were 10 and 100 times their respective inhibition constants (K_i) (obtained from *in vitro* studies^{23,25}). As the K_i for sphingosine is unknown, we used concentrations of 50 μM and 200 μM , the latter reducing protein kinase C activity by 90% in several cell types^{24,25}. The inhibitors had no significant effect on the OAG responses. All values were obtained a minimum of 10 min after the start of cell perfusion and, in several cells, after 30 min. With 2 mM ATP and 40 mM HEPES buffer in the internal pipette, there was no evidence for rundown of the Ca^{2+} current before 30 min (see also refs 27 and 28). Similarly, internal H-7 (50 μM) had no effect on the diC_8 or phorbol ester responses (Table 1, centre). Sphingosine and H-7, applied at the same concentration to the outside of cells, quickly and reversibly reduced the Ca^{2+} current (Table 1, bottom).

In a different approach, we tested whether OAG was effective if applied intracellularly. For these experiments, we used two

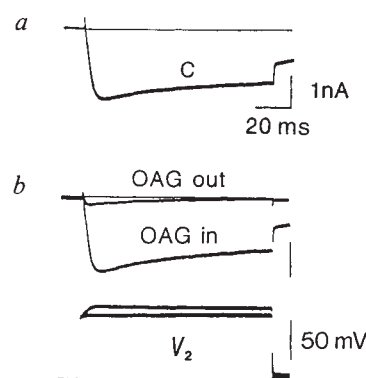


FIG. 3 Current and voltage measurements using a three-electrode recording arrangement to compare the effects of OAG applied inside (50 μM) and outside (5 μM) to the same cell. *a*, Control record (*c*) of the Ca^{2+} current recorded with the first patch electrode during a voltage step from -80 mV to 0 mV. *b*, 10 min after the whole-cell configuration was established with the second (OAG) patch electrode, the Ca^{2+} current was unchanged (OAG in). External application of OAG through a third microelectrode was still effective at blocking the Ca^{2+} current (OAG out) however. Membrane voltage with the second patch electrode (V_2) was recorded in the current-clamp mode. The slowly decaying tail currents in the control and internal OAG records may indicate inadequate clamping on repolarization as they were not present under better recording conditions¹³.

patch pipettes, one filled with control internal saline and the other with internal saline plus 50 μ M OAG (Fig. 3). This high concentration was necessary because OAG is rapidly metabolized¹⁵. In all seven experiments we found no change in the Ca^{2+} current during intracellular OAG application even after 25 min (average duration was 15 min). In five cases, we applied OAG externally from a third electrode and recorded the usual OAG decrease of the current (Fig. 3b). In three similar experiments using H-7 (50 μ M) in the second electrode instead of OAG, there was no measurable effect on the Ca^{2+} current. Because micromolar Ca^{2+} concentrations are necessary for protein kinase C activity in the absence of diacylglycerol analogues¹, total CaCl_2 concentration in the internal solution was raised for these experiments from 1 mM to 9.1 mM to increase the free Ca^{2+} concentration from 0.1 μ M to 1 μ M.

We conclude that OAG and H-7 act on the outside of dorsal root ganglion neurons to depress the Ca^{2+} current. We suspect that the other agents tested act in the same way, although our evidence is less direct. At the inhibitor concentrations used, both protein kinase C and cyclic AMP-dependent and cyclic GMP-dependent protein kinases should have been completely inhibited, indicating that the functioning of Ca^{2+} channels in dorsal root ganglion neurons does not depend on these protein kinases. This might not be the case in all cell types²⁹⁻³¹.

The demonstration of external action of activators and inhibitors of protein kinase C is reminiscent of recent results for forskolin, an adenylyl cyclase activator. Several studies have presented evidence that forskolin may affect Ca^{2+} currents³² and K^{+} currents³³⁻³⁶ independently of cyclase activity. Like forskolin, OAG and H-7 did not affect the Ca^{2+} current when applied intracellularly. These molecules apparently possess little affinity for the Ca^{2+} channel on the inner side of the membrane, although the fast on-kinetics suggest a high-affinity external binding site. Direct antagonistic effects on Ca^{2+} channels must be considered when applying these agents extracellularly to study events involving protein kinase C. $\square$

Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome

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GERSTMANN-Sträussler syndrome is a rare familial neurodegenerative condition that is vertically transmitted, in an apparently autosomal dominant way¹. It can also be horizontally transmitted to non-human primates and rodents through intracerebral inoculation of brain homogenates from patients with the disease²⁻⁸. The exact incidence of the syndrome is unknown but is estimated to be between one and ten per hundred million. Patients initially suffer from ataxia or dementia and deteriorate until they die, in one to ten years. Protease-resistant prion protein (PrP) and PrP-immunoreactive amyloid plaques with characteristic morphology accumulate in the brains of these patients⁹⁻¹¹. Current diagnostic criteria for Gerstmann-Sträussler syndrome incorporate clinical and neuropathological features, as animal transmission studies can be unreliable^{8,12}. PrP is implicated in the pathogenesis and transmission of the condition and in scrapie, an equivalent animal disease¹³⁻¹⁷. It was discovered by enriching scrapie-infected hamster brain fractions for infectivity^{18,19}. Because there is compelling evidence that the scrapie isoform of PrP is a necessary component of the infectious particle^{15,16}, it seemed possible that the PrP gene on the short arm of human chromosome 20 (ref. 20) in Gerstmann-Sträussler syndrome might be abnormal. We show

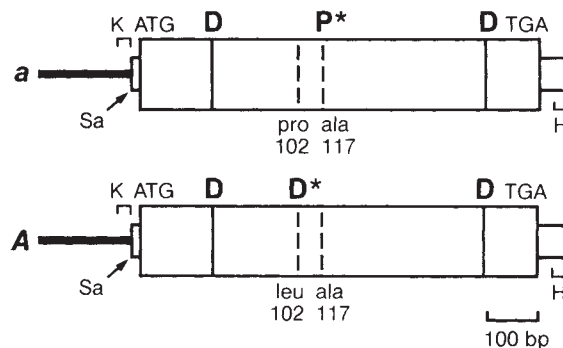


FIG. 1 Organization of PrP alleles in ataxic GSS. Top, Wild-type PrP allele (a); bottom, variant PrP allele found in ataxic GSS (A). The open reading frame is indicated by an open box, the mRNA untranslated region by a narrower box and the intron by a solid black line. Restriction sites: P, *PvuII*; D, *DdeI*. Sa indicates a putative 3' splice site (splice acceptor). Asterisks indicate locations of polymorphic restriction sites; broken vertical lines indicate locations of polymorphic codons. Brackets indicate locations of synthetic primers K (5'-AAGAATTCTCTGACATTCTCCTCTTCA-3') and H (5'-AAGATCCCTCAAGCTGAAAAAG-3') used in cyclic thermal DNA amplifications. The K oligonucleotide is based on unpublished sequence data of the intron in the human PrP gene; a partial sequence of the intron 5' to the open reading frame of the A allele and including the initiation codon is: 5'-CATTATGCAGAAACATT-AGTAATTCACATAAATATGGAACCTGACATTCTCCTCTTCTTTCGACAGCA-GTCATTATG-3'. This data was obtained during sequencing of the two PrP alleles in patient JJ. H and K oligonucleotides were constructed to include 5'-*Bam*HI and *Eco*RI linkers, respectively.

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here that PrP codon 102 is linked to the putative gene for the syndrome in two pedigrees, providing the best evidence to date that this familial condition is inherited despite also being infectious, and that substitution of leucine for proline at PrP codon 102 may lead to the development of Gerstmann-Sträussler syndrome.

We sequenced both alleles of the PrP open reading frame in J.J., a patient with Gerstmann-Sträussler syndrome (GSS) from a US pedigree (see III-a, Fig. 2a). Five recombinant bacteriophage containing the PrP open reading frame were recovered from a λ -EMBL-3 library constructed from the patient's peripheral leukocyte DNA and screened with a human PrP cDNA²¹. There are two PrP alleles, which can easily be distinguished in genomic DNA and recombinant clones because one allele differs from the other by the absence of a *PvuII* restriction site located in the PrP open reading frame. This polymorphism has been reported previously; the *PvuII* site is present in 90% of the population²². We found that the *PvuII* site was abolished by a 'silent' adenine to guanine substitution in the third position of alanine codon 117. One of the five clones lacked this *PvuII* site. In the same clone, a cytosine to thymine substitution in the second position of codon 102 resulted in a proline to leucine change, creating a *DdeI* restriction site (Fig. 1). No other variations between the two alleles were found. The other allele was identical to another published PrP gene sequence²¹ and is presumably the wild-type allele.

To determine whether the leucine substitution at codon 102 was present in other individuals we used either Southern blotting analysis with *DdeI*-digested genomic DNA, or restriction digests of genomic DNA amplified by the polymerase chain reaction (Fig. 1) visualized directly with ethidium bromide (see Fig. 3). We found the leucine substitution in the two other individuals (J.B. and J.C.) that we examined with ataxic GSS, both of whom are members of an extensive British pedigree (see III-20 and

IV-2, Fig. 2, lower pedigree). The leucine substitution was not present in eight unaffected relatives of J.J., J.B., or J.C. who are beyond the age for being at risk for the disease (Figs 2 and 3). Nor was it found in 15 patients with other forms of inherited or sporadic prion diseases, or in 100 unrelated, normal Caucasian individuals of the same ethnic background as the British and US pedigrees.

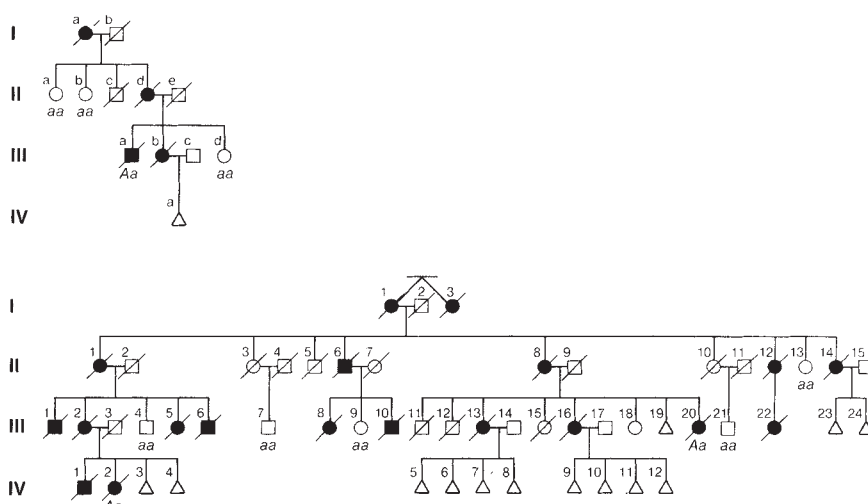
The family data were analysed for genetic linkage using the LIPED computer program²³. We assumed that the disease followed a dominant mode of inheritance with age-dependant penetrance, where penetrance was assumed to rise linearly from 0 at age 21 to 100% at age 40 in the US family and from 0 at age 38 to 100% at age 66 in the British family. Under these conditions, the log likelihood ratio (lod score) is lowered by asymptomatic individuals with the leucine substitution who are older than the minimum age of onset. As the disease is known to be extremely rare, a population frequency of $q=1$ in 10,000,000 was assumed for the disease gene, corresponding to a disease prevalence of 2 in 10,000,000 among older individuals beyond the age at risk. Even for values of q as high as 0.0001, the results in Table 1 are unaltered.

The population frequency of the leucine substitution, p , is crucial for the linkage analysis as many married-in spouses are of unknown genotype at this marker locus. When the substitution is rare, it is probably inherited from one of the founding members in each pedigree who also passed the disease gene to his or her offspring. None of a random sample of 100 Caucasian individuals carried the substitution (no substitution in 200 alleles) so that the 95% confidence interval for the allele frequency, p , extends from 0 to $1 - 0.05^{0.005} = 0.015$ and the 99% confidence interval for p ranges from 0 to 0.023.

The results shown in Table 1 demonstrate significant tight linkage between the disease and the marker locus, the maximum

FIG. 2 Pedigrees of two families with ataxic GSS. Circles, females; squares, males; triangles, males or females; a black symbol indicates that an individual is affected with GSS; a slashed symbol that an individual is deceased. The clinical and pathological aspects of these pedigrees will be described in detail elsewhere. On the basis of the predicted amino-acid sequence, *a* indicates a proline at codon 102 and *A* indicates a leucine at that codon. Because of their predictive nature, the alleles for individuals at risk for illness (triangles) are not shown in order to protect the confidentiality of these people. The distribution of the candidate *A* alleles in the at-risk group was found to be in accordance with that expected for an autosomal dominant trait. Detailed information of allelic assignments on which the lod score calculations are based will be made available to investigators for scientific purposes only but in strict confidence. Top, Pedigree of US family with ataxic GSS. This is a previously unreported pedigree. IV-a (age 23) developed leg pains and gait instability in 1988,

but is scored as an individual at risk for illness in the absence of a more definitive diagnosis. The diagnosis of GSS was confirmed in III-a and III-b by positive staining of amyloid plaques with anti-PrP antibodies. The known ages (in years) of onset for affected individuals are: I-a, 40; II-d, 22; III-a, 39; and III-b, 29. Hamsters which received intracerebral inoculations of a 10% brain homogenate from III-a are still asymptomatic after 500 days. Both alleles of the PrP open reading frame were sequenced in the index case, III-a, using a combination of methods described by Maxam and Gilbert³⁵ and Biggin and co-workers³⁶. Bottom, Pedigree of a British family with GSS. This pedigree has been extensively analysed, both clinically and pathologically^{4,6,37,38}. PrP-positive plaques were demonstrated in III-20 and IV-2. The known ages (in years) of onset for affected individuals are: II-6, 52; II-8, 46; II-12, 66; III-1, 65; III-2, 54; III-5, 57; III-6, 60; III-8, 48; III-10, 42; III-13, 51; III-16, 39; III-20, 48; III-22, 59; and IV-2, 40. The ages of individuals at risk for illness are: III-19; 58; III-23, 58; III-24, 51; IV-3, 53; IV-4, 51; IV-5, 38; IV-6, 34; IV-7, 32; IV-8, 30; IV-9, 40; IV-10, 39; IV-11, 35; and IV-12, 34. Hamsters which received intracerebral inoculations of 10% brain homogenates from III-20 and IV-2



developed neurological disease at 317 ± 21.8 ($n=5$) and 300 ± 11.2 ($n=8$) days, respectively, and died at 327 ± 19.6 and 305 ± 11.5 days, respectively. Transmission of brain homogenates from IV-2 (ref. 6) and III-10 (ref. 4) to monkeys has been reported previously.

METHODS. Relevant family members were examined by a neurologist and blood samples were obtained. DNA was extracted from the buffy coat in all instances except IV-2 for which only frozen brain was available. DNA from III-20 and IV-2 was extracted from freshly frozen tissue. We also extracted DNA using a previously published method³⁴ from formalin-fixed brains from several other affected members of these two pedigrees, for example III-b, III-10 and III-22, and from a single patient from a third pedigree. We found discrepancies between data obtained from matched control samples of DNA from fresh and formalin-fixed tissue, however. Therefore, even though the mutant *DdeI* site was present in each of these four additional GSS cases, we excluded these data from our analysis. Codon 102 of the PrP open reading frame was determined by digestion with *DdeI* of DNA amplified between the primers described in Fig. 1.

lod score being equal to $Z = 3.26$ at a recombination fraction of $\Theta = 0$ ($p = 0.01$). The commonly used approximate confidence interval for Θ extends from 0 to 0.14, which is the value of Θ with a lod score of $Z - 1$.

The US and British pedigrees seem to be lineally distinct both on the basis of currently available genealogical inquiries and comparison of the restriction map in the PrP open reading frame. The *PvuII* polymorphism at codon 117 is present in the US family but not in the British family. Although more extensive genealogical investigation could conceivably reveal common ancestry between these two pedigrees, at present it seems more likely that the mutation causing the leucine substitutions at codon 102 arose independently in the two pedigrees. This base substitution may have involved deamination of a methylated cytosine situated 5' to guanine and therefore represents a relatively common mutation in human DNA²⁴. The proline at codon 102 seems to be highly conserved as all rodent PrP genes sequenced so far also encode a proline at the equivalent codon^{14,24-29}.

The codon 102 proline→leucine substitution is possibly located within a surface loop which is nine residues from a putative transmembrane α -helix³⁰. It would therefore be unlikely to exert a significant effect on the secondary structure of PrP. The biological consequences of single amino-acid substitutions that do not perturb secondary structure can nevertheless be profound, as in certain haemoglobinopathies and neoplasias³¹⁻³³.

We believe that, on the basis of clinical and pathological criteria, GSS can be classified into three forms: an ataxic form, for which the leucine substitution seems to be characteristic; a dementing form; and a dementing form that is accompanied by pathological quantities of neurofibrillary tangles. When familial Creutzfeldt-Jakob disease is included, at least four forms of human inherited prion diseases can be deciphered⁴⁰. A fuller

TABLE 1 Lod scores for the two families for several values of p , the leucine substitution frequency

p	Recombination fraction				
	0	0.05	0.10	0.15	0.20
0.023	0.634	0.521	0.411	0.308	0.215
	<u>2.471</u>	<u>2.238</u>	<u>1.989</u>	<u>1.723</u>	<u>1.441</u>
	3.105	2.759	2.400	2.031	1.656
0.015	0.640	0.526	0.415	0.311	0.218
	<u>2.562</u>	<u>2.328</u>	<u>2.077</u>	<u>1.809</u>	<u>1.524</u>
	3.202	2.854	2.492	2.120	1.742
0.01	0.643	0.529	0.418	0.313	0.219
	<u>2.617</u>	<u>2.382</u>	<u>2.130</u>	<u>1.860</u>	<u>1.573</u>
	3.260	2.911	2.548	2.173	1.792
0.001	0.650	0.534	0.422	0.317	0.222
	<u>2.707</u>	<u>2.471</u>	<u>2.218</u>	<u>1.946</u>	<u>1.655</u>
	3.357	3.005	2.640	2.263	1.877
0.0001	0.650	0.535	0.423	0.317	0.222
	<u>2.715</u>	<u>2.479</u>	<u>2.225</u>	<u>1.953</u>	<u>1.663</u>
	3.365	3.014	2.648	2.270	1.885
10^{-7}	0.650	0.535	0.423	0.317	0.222
	<u>2.717</u>	<u>2.480</u>	<u>2.227</u>	<u>1.954</u>	<u>1.664</u>
	3.367	3.015	2.650	2.271	1.886

The format is: lod scores for the US family + lod scores for the British family = summed lod scores for both families.

description of this proposed classification will be published elsewhere.

The linkage between the PrP gene and GSS in two pedigrees establishes, for the first time, that GSS is a genetic disorder. Our observations parallel earlier studies where the murine PrP gene was found to be linked to susceptibility (that is, length of the incubation time) for experimental scrapie after inoculation with prions¹³. Moreover, our discovery of a unique PrP gene amino-acid substitution on the same chromosome that carries the GSS locus in two pedigrees also resembles earlier studies in which substitutions of two amino-acid residues at PrP codons 108 and 189 correlate with short and long incubation times in experimental scrapie studies in mice¹⁴. If the leucine substitution at codon 102 arose independently in our two pedigrees, then we believe it could represent more than a linked genetic marker and might itself provoke the development of GSS. Although the composition of the GSS prion has not been determined directly, it seems likely, by analogy with results from experimental scrapie studies in rodents, that the GSS prion is composed largely, if not entirely, of an abnormal isoform of human PrP. The striking and specific correlation between the leucine substitution and the ataxic form of GSS leads us to speculate that other specific mutations in PrP may correlate with the other forms of inherited prion diseases. □

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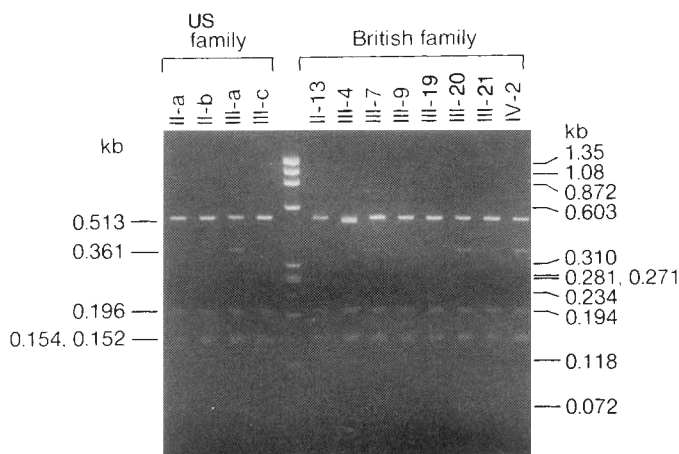


FIG. 3 Restriction patterns of twelve informative cases from the families with ataxic GSS shown in Fig. 2.

METHODS. Genomic DNA extracted from peripheral leukocytes was amplified using the *Thermus aquaticus* (Taq) heat-stable DNA polymerase as previously described³⁹. Amplification reactions used 1-2 μ g of DNA in a 100 μ l solution containing 1 μ M each of primer KH10 and KH11 and 2.5 units Taq polymerase in the reaction buffer provided by the supplier (Perkin-Elmer-Cetus). The samples were overlaid with two drops of mineral oil to prevent evaporation and were subjected to 35 cycles of amplification. The cycling reaction was performed in a programmable heat block (DNA Thermal Cycler, PECO) set to incubate the samples at 94 °C for 90 s (to denature the DNA), at 55 °C for 60 s (to anneal the primers), and at 72 °C for 3 min (to extend the annealed primers). The reaction product (30-60 μ l) was fractionated on a preparative 1.5% agarose gel from which an 864-base pair fragment was excised and purified using GeneClean (Bio 101). The excised fragment (30 mg) was digested to completion with 5 units *DdeI* in a volume of 12 μ l using the buffer recommended by the supplier. The DNA was fractionated on a 3%:1% NuSieve-SeaKem agarose gel (FMC Corp.) containing ethidium bromide (0.5 μ g ml⁻¹) in 40 mM Tris-acetate, 2 mM EDTA buffer. *HaeIII*-digested ϕ X 174 DNA (250 ng) was loaded as a size marker.

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Polymorphism in the α_3 domain of HLA-A molecules affects binding to CD8

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CYTOTOXIC T lymphocytes (CTL) expressing the CD8 glycoprotein recognize peptide antigens presented by class I major histocompatibility complex (MHC) molecules^{1,2}. This correlation and the absence of CD8 polymorphism led to the hypothesis that CD8 binds to a conserved site of class I MHC molecules. Using a cell–cell binding assay we previously demonstrated specific interaction between human class I MHC (HLA-A,B,C) molecules and CD8 (ref. 3). Subsequent analysis of the products of 17 HLA-A,B alleles revealed a natural polymorphism for CD8 binding in the human population. Two molecules, HLA-Aw68.1 and HLA-Aw68.2, which do not bind CD8, have a valine residue at position 245 whereas all other HLA-A,B,C molecules have alanine. Site-directed mutagenesis shows that this single substitution in the α_3 domain is responsible for the CD8 binding phenotype and also affects recognition by alloreactive and influenza-specific CTL. Our results indicate that CD8 binds to the α_3 domain of class I MHC molecules.

Various class I MHC alleles were transfected into the HLA-A,B negative B-cell line, CIR. The resulting transfectants were tested for binding to Chinese hamster ovary (CHO) cells expressing the human CD8 gene, using the assay described by Norment *et al.*³. Fifteen of 17 HLA-A,B molecules tested gave significant levels of positive binding, as did two murine class I MHC

TABLE 1 Binding of CD8 to class I molecules

Experiment	Transfected class I gene	Cells bound per well ($\times 10^{-3}$) CD8 ⁺	CD8 ⁻
I	HLA-A2.1	32.2 $\pm$ 6.4	8.8 $\pm$ 0.8
	A2.2Y	38.8 $\pm$ 1.0	11.2 $\pm$ 1.0
	A2.3	33.1 $\pm$ 1.9	9.5 $\pm$ 1.2
	Aw69	25.6 $\pm$ 3.0	12.5 $\pm$ 0.9
	A3.1	49.6 $\pm$ 1.2	9.2 $\pm$ 1.8
	A3.2	31.4 $\pm$ 0.6	5.3 $\pm$ 0.4
	A1	27.2 $\pm$ 1.4	9.7 $\pm$ 2.1
	A24	28.3 $\pm$ 1.6	6.5 $\pm$ 1.2
	Aw68.1	15.1 $\pm$ 1.8	8.2 $\pm$ 1.3
	Aw68.2	13.9 $\pm$ 1.0	9.7 $\pm$ 1.3
	untransfected CIR	18.2 $\pm$ 1.1	16.6 $\pm$ 1.6
II	HLA-B13	60.4 $\pm$ 4.6	25.3 $\pm$ 2.3
	B38	41.1 $\pm$ 2.9	10.6 $\pm$ 0.6
	B44.2	54.4 $\pm$ 2.1	21.1 $\pm$ 1.2
	B49	41.1 $\pm$ 3.8	11.5 $\pm$ 1.2
	B51	41.7 $\pm$ 0.4	15.5 $\pm$ 1.2
	untransfected CIR	26.1 $\pm$ 0.3	23.4 $\pm$ 0.5
III	HLA-Bw58	39.6 $\pm$ 4.7	8.1 $\pm$ 2.1
	HLA-B7.1	20.2 $\pm$ 0.8	5.7 $\pm$ 1.2
	H-2D ^p	60.2 $\pm$ 9.7	11.7 $\pm$ 2.8
	H-2K ^b	86.7 $\pm$ 6.0	8.8 $\pm$ 5.9
	untransfected CIR	17.9 $\pm$ 2.2	14.1 $\pm$ 1.5

All transfectants were assayed as described in Fig. 1 legend. Results of three experiments are shown. The binding of class I transfected and untransfected CIR cells to CD8 expressing (CD8⁺) and non-expressing (CD8⁻) CHO cells is shown. Only HLA-Aw68.1 and HLA-Aw68.2 reproducibly showed no specific binding to CD8. The significance of variation in the positive binding of the other class I molecules is not known; it may result from differences in the properties of the individual transfectants and/or the affinity of the class I–CD8 interaction.

molecules, H-2K^b and H-2D^p (Table 1). These results indicate conservation of the CD8 binding site in class I molecules of humans and mice. It was therefore surprising to find that two human class I molecules; HLA-Aw68.1 and -Aw68.2, showed no specific interaction with CD8 in the cell–cell binding assay (Table I). This effect cannot be attributed to differing levels of HLA-A expression by the various transfectants (Fig. 1) and most probably results from differences in their primary structures. Fortunately, HLA-Aw68.1 and -Aw68.2 are closely related in amino-acid sequence to molecules such as HLA-A2.1 and HLA-Aw69 which do bind CD8. For example, HLA-Aw69 and HLA-Aw68.1 differ at only six positions in the α_2 domain and one position (245) in the α_3 domain⁴. We therefore reasoned that one or more of these substitutions is responsible for the difference in CD8 binding. Furthermore, a comparison of over 50 HLA-A,B,C sequences identified 245 as the only position at which there is a substitution that is specific to HLA-Aw68.1 and -Aw68.2 (ref. 4 and unpublished observations). In these two molecules, Val occurs at position 245 whereas other HLA-A,B,C molecules have Ala. These considerations suggested the involvement of residue 245 and the α_3 domain in the CD8 binding site.

To test this hypothesis we made a pair of mutants: the Ala 245 of HLA-A2.1 was converted to Val, giving the A2.1m245 mutant; and the Val 245 of HLA-Aw68.1 was converted to Ala, giving the Aw68.1m245 mutant. Analysis of transfectants expressing these mutant genes showed that the CD8 binding phenotype correlated with the residue at position 245 (Fig. 1). Thus mutant A2.1m245 showed no binding to CD8 whereas mutant Aw68.1m245 bound CD8 equivalent to wild-type HLA-A2.1. The specificity of this binding was also identical to that obtained with wild-type HLA-A2.1, in that it was dependent on CD8 and could be inhibited by monoclonal antibodies against CD8 and class I HLA molecules. Results of similar experiments with various other mutants argue against the involvement of the α_1 and α_2 domains in binding CD8. In these mutants the six

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TABLE 2 CD8 binding to mutants of HLA-Aw69 and Aw68.1

HLA molecule	Amino-acid position							CD8 binding
	95	97	107	114	116	156	245	
Aw69	V	R	W	H	Y	L	A	+
69m95, 97, 245	I	M	W	H	Y	L	V	—
69m107, 245	V	R	G	H	Y	L	V	—
68.1m95, 97	V	R	G	R	D	W	V	—
68.1m107	I	M	W	R	D	W	V	—
Aw68.1	I	M	G	R	D	W	V	—

HLA-Aw69 and Aw68.1 differ only at the seven positions shown. Amino-acid substitutions in the HLA mutants are given by the standard single-letter code. Assays were performed as described in Fig. 1 legend.

differences (positions 95, 97, 107, 114, 116, 156) between HLA-Aw69, which binds CD8, and HLA-Aw68.1, which does not, were assessed. Conversion of various combinations of these substitutions from one sequence to the other had no effect on the CD8 binding phenotype, which in every case correlated with residue 245 (Table 2).

Previous speculation has suggested an Arg-Phe-Asp-Ser (RFDS) sequence, shared by many class I and class II MHC sequences and related to the fibronectin cell binding tetrapeptide Arg-Gly-Asp-Ser (RGDS), as a possible site for CD8 or CD4 binding⁵. Furthermore, Auffray *et al.* have shown that RFDS-containing peptides derived from class II sequences inhibit T-cell responses⁶. Conversely, the RFDS sequence of HLA-A2.1 (residues 35–38 of the α_1 domain) is, from the crystallographic structure, inaccessible and therefore an unlikely site for CD8 binding^{7,8}. This view is supported by our finding that a mutant of HLA-A2.1 in which Arg 35 is substituted by Val shows no loss of CD8 binding activity (data not shown).

The question arises as to whether the differences in the cell-binding assay have functional consequences for cytotoxic T cells. To address this, we compared recognition of position 245 mutants by both alloreactive and antigen-specific, MHC-

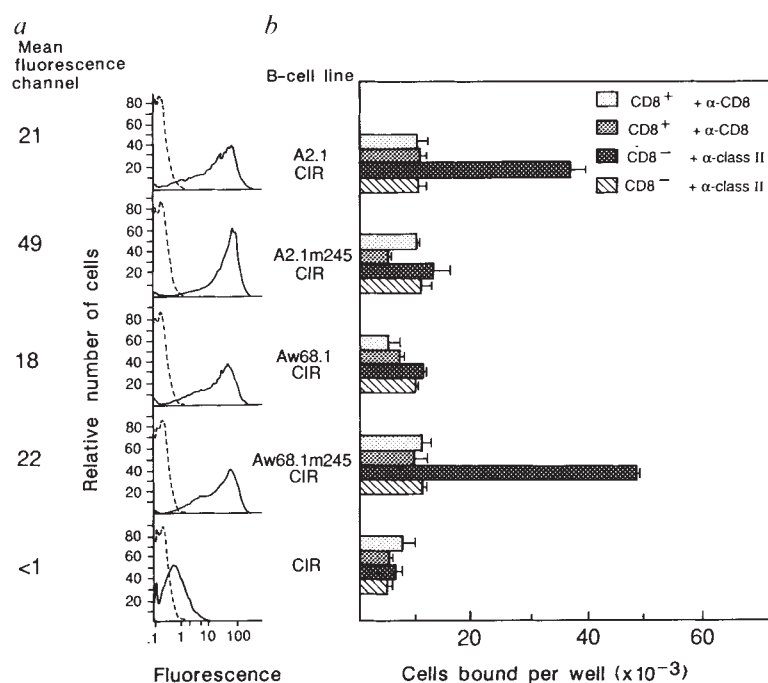
restricted CTL. The alloreactive CTL show specificity for HLA-A2.1 and effectively lyse the HLA-A2.1 transfectant. This lysis, however, is reduced by replacement of Ala 245 with Val (Fig. 2). By contrast, these CTL show a poor lysis of the HLA-Aw68.1 transfectant, which is considerably improved by replacement of Val 245 with Ala. Similar effects were observed with HLA-A2.1-restricted CTL lines specific for peptide 56–68 of the influenza matrix protein. Cytolysis was significantly reduced when Ala 245 of the HLA-A2.1 restriction molecule was replaced by Val (Fig. 2). Changes in CTL function can thus be correlated with changes observed in the cell-cell binding assay: greater lysis is observed when the target molecule binds more strongly to CD8.

Both alloreactive and antigen-specific CTL used in these experiments were inhibited by anti-CD8 monoclonal antibodies. Surprisingly, inhibition was not dependent upon whether the class I target could bind CD8 in the cell-cell binding assay. Thus recognition of HLA-Aw68.1 or HLA-A2.1m245 was inhibited by anti-CD8. In representative experiments, cytolysis by alloreactive CTL of HLA-Aw68.1 and HLA-A2.1 was, respectively, reduced from 29 to 8% and from 56 to 39% by anti-CD8 antibody 51.1. Lysis of peptide-sensitized A2.1m245 transfectants by influenza-specific CTL was reduced from 37 to 16% by anti-CD8, whereas no effect was seen on lysis of HLA-A2.1 transfectants. These observations might reflect a residual affinity of these molecules for CD8 that is not detected in the cell binding assay. An alternative is suggested by other studies in which the inhibition of CTL by anti-CD8 antibodies, in a manner that is unrelated to binding of class I molecules, is interpreted as evidence for a regulatory role for CD8 in T-cell activation^{9,10}.

It is clear that substitution at position 245 alters the interaction of class I HLA molecules with CD8. This could result from a direct involvement of this residue in the interaction or by an indirect conformational effect on a distant site. The crystallographic structures of HLA-A2.1 and -Aw68.1 at high resolution have been determined and they are extremely similar (T. Garrett, M. Saper, P. Bjorkman and D. Wiley, unpublished observations). Thus, it is probable that the distinct functional properties

FIG. 1 Substitution at position 245 determines natural polymorphisms in CD8 binding. *a*, Expression of HLA-A2.1, A2.1m245, Aw68.1m245 and Aw68.1 molecules by transfectants of the CIR B-cell line which expresses no endogenous HLA-A,B molecules¹⁸. Indirect binding of the HLA-A2,A28-specific monoclonal antibody CR11-351 (ref. 19) (solid lines) was compared to an anti-actin control²⁰ (dotted lines) by flow cytometry. *b*, Binding of class I transfectants to CD8 expressing and non-expressing CHO cells in the presence of either anti-CD8 or anti-HLA-DR. Binding to CHO.1 and CHO.4 cells is labelled CD8⁺ and CD8[−], respectively. In each group of four results the combination of cells and antibodies is, going from top to bottom: CD8⁺ CHO cells with anti-CD8 (α -CD8); CD8[−] CHO cells with anti-CD8; CD8⁺ CHO cells with anti-HLA-DR (α -class II); CD8[−] CHO cells with anti-HLA-DR. The standard errors are indicated by the 'error' bars. The HLA-A2,A28-specific monoclonal antibody CR11-351 also inhibited cell-cell binding when preincubated with the CIR transfectants (data not shown). A second independently derived set of transfectants had identical CD8 binding characteristics (data not shown).

METHODS. Mutagenesis and transfection of class I genes were as previously described¹⁸. Flow-cytometric analysis of cells incubated first with monoclonal antibody and then with fluoresceinated goat anti-mouse antibody used a Becton-Dickinson FACS with a 10 $\times$ neutral density filter. In CD8 binding experiments, CIR transfectants with class I genes were radio-labelled with ³⁵S methionine, washed twice, resuspended in PBS-containing, 10% fetal calf serum, and incubated in 96-well plates with confluent monolayers of transfected CHO cells. The CHO cells were previously incubated (30 min, 20 μ g ml^{−1}, 37°) either with monoclonal antibody 51.1 (ref. 21), which is specific for CD8, or CA206, which is specific for HLA-DR (ref. 22), and was used as an irrelevant antibody control. CHO.1



cells express no CD8 whereas CHO.4 cells express ~160-fold more CD8 than peripheral T cells³. After incubation at 37° for 1 hour, wells were washed extensively, and bound radioactivity measured.

of these two molecules arise from the nature and disposition of the side-chains at the positions of amino-acid substitution and not through gross conformational differences. These observations combined with the complete change in CD8 binding phenotype caused by single substitutions at position 245 in HLA-A2.1 and HLA-Aw68.1 strongly favour the direct involvement of this residue and the α_3 domain in the CD8 binding site.

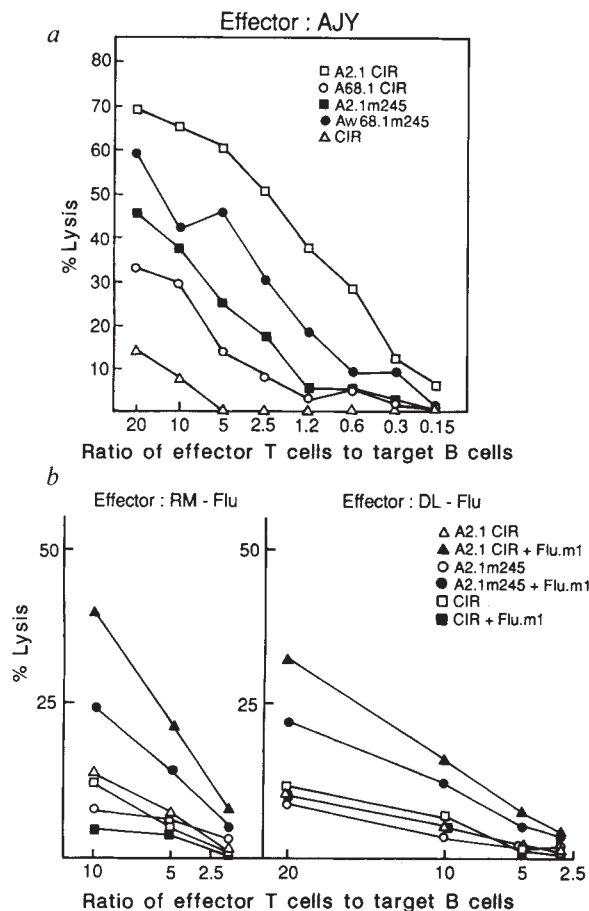


FIG. 2 Cytolysis of class I HLA-expressing CIR transfectants by alloreactive and antigen-specific T cells. *a*, Lysis of CIR transfectants expressing HLA-A2.1, A2.1m245, Aw-68.1m245 and Aw68.1 by an HLA-A2 alloreactive CTL culture AJY. In some experiments, the A2.1m245 transfectants were lysed better than Aw68.1m245-bearing cells. In all experiments, however, the AJY culture recognized HLA-A2.1 more effectively than A2.1m245, and Aw68.1m245 more effectively than HLA-Aw68.1. *b*, Lysis by influenza-specific CTL of HLA-A2.1- and A2.1m245-expressing transfectants after incubation with peptide corresponding to residues 56–68 of the influenza matrix protein. Donor RM (HLA-A1,A2; Bw55, B44), left panel; donor DL (HLA-A2, A11; B27, B44), right panel. All but one of the six donors gave strong influenza-specific, HLA-A2.1-restricted CTL. None of the responders lysed HLA-Aw68.1- or Aw68.1m245-bearing transfectants. $\blacktriangle$, $\triangle$; lysis of A2.1 transfectant with and without the Flu.m1 peptide, respectively. $\bullet$, $\circ$; lysis of A2.1m245 transfectant with and without the Flu.m1 peptide, respectively. $\blacksquare$, $\square$; lysis of untransfected CIR cells with and without the Flu.m1 peptide, respectively. METHODS. ^{51}Cr -release assays were performed as previously described²³. The AJY culture was generated by *in vitro* stimulation of peripheral blood lymphocytes (PBL) from a single donor AK (HLA-A3; B7, B38) with the HLA-A2.1-expressing cell line JY (ref. 23), and it lyses HLA-A2.1-, Aw69-, and Aw68-bearing targets with varying efficiency. Influenza-specific cultures were generated essentially as previously described²⁴. PBL from six HLA-A2 positive donors were stimulated with $10 \mu\text{g ml}^{-1}$ of a peptide (Flu.m1) derived from residues 56–68 of the influenza matrix protein shown previously to generate HLA-A2-restricted CTL (ref. 25). Three days after the initial stimulation, supernatant containing growth factors from IL-2-stimulated PBL was added. Cultures were restimulated after 7 days to generate secondary cultures. For cytolysis assays, target cells were preincubated overnight with $10 \mu\text{g ml}^{-1}$ of the Flu.m1 peptide, then used in ^{51}Cr -release experiments.

Further support for this interpretation comes from experiments showing that a mutant of H-2D^d derived *in vitro* with a single substitution at position 227 shows specific loss of interaction with cytotoxic T cells that are dependent on Lyt-2, the murine equivalent of CD8^{11,12}. Positions 227 and 245 of the α_3 domain are located proximal to the membrane attachment site in a solvent accessible surface region and are separated by only 10.5 Å (refs 7, 8). Both could therefore contribute to the CD8 binding site.

Davis and Bjorkman have raised the possibility that CD8 interacts with the α_1 and α_2 domains of class I MHC molecules, in a manner analogous to the T-cell receptor¹³. A consequence of this model is that simultaneous interaction of CD8 and the T-cell receptor with the same class I MHC molecule cannot occur. Our results and those of Potter *et al.*^{11,12}, suggesting interaction of CD8 with the α_3 domain, do not support this view and leave open the possibility that a complex of T-cell receptor and CD8, binding to the same class I MHC molecule (plus its antigenic peptide), is crucial to T-cell activation.

Comparison of the amino-acid sequences of antigen-presenting class I MHC molecules from seven mammalian species (summarized in ref. 14) shows that HLA-Aw68.1 and -Aw68.2 are unique in not having Ala at position 245. This combined with the observation that all 15 molecules with Ala at this position bound CD8 in the cell-cell binding assay suggest that the non-binding CD8 phenotype will be rare among class I MHC products. In most human populations, however, the frequency of HLA-A28, of which HLA-Aw68.1 and -Aw68.2 are subtypes, is 8–9% (ref. 15). The intriguing question is whether reduced CD8 binding confers any selective advantage or disadvantage to individuals expressing these molecules. For example, does it affect the capacity of HLA-Aw68.1 and -Aw68.2 to present antigens, or the repertoire of HLA-Aw68.1- and HLA-Aw68.2-restricted T-cell receptors selected in the thymus? In studies by Rickinson *et al.* CTL specific for Epstein-Barr virus (EBV), generated by *in vitro* priming and restricted to HLA-Aw68, were never obtained, although HLA-Aw69-restricted CTL lysed infected HLA-Aw68 targets^{16,17}. This suggests that although HLA-Aw68 can present EBV peptides to specific CTL, it may be deficient in generating such a response. The number of epitopes and restriction patterns described for human class I MHC molecules, however, is still too small to assess the relative use of HLA-Aw68.1 and -Aw68.2. $\square$

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Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification

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THE molecular analysis of many genetic diseases requires the isolation of probes for defined human chromosome regions. Existing techniques such as the screening of chromosome-specific libraries¹, subtractive DNA cloning² and chromosome jumping³ are either tedious or not generally applicable. Microdissection and microcloning has successfully been applied to various chromosome regions in *Drosophila* and mouse⁴⁻⁹, but conventional microtechniques are too coarse and inefficient for analysis of the human genome¹⁰⁻¹². Because microdissection has previously been used on unbanded chromosomes only, cell lines in which the chromosome of interest could be identified without banding had to be used. At least one hundred chromosomes were needed for dissection and λ vectors used to achieve maximum cloning efficiency. Recombinant phage clones are, however, more difficult to characterize than plasmid clones. Here we describe the dissection of the Langer-Giedion syndrome region on chromosome 8 from GTG-banded metaphase chromosomes (G-banding with trypsin-Giemsa) and the universal enzymatic amplification of the dissected DNA. Eighty per cent of clones from this library (total yield 20,000) identify single-copy DNA sequences. Fifty per cent of clones detect deletions in two patients with Langer-Giedion syndrome. Although the other clones have not yet been mapped, this result demonstrates that thousands of region-specific probes can be isolated within ten days.

We have isolated probes for the Langer-Giedion syndrome chromosome region (8q24.1) (refs 13, 14). Deletion of a set of unknown genes within this region leads to sparse hair, lax skin, bulbous nose, cone-shaped epiphyses, multiple cartilaginous exostoses and mental retardation¹⁵. Using an electronically controlled micromanipulator, we dissected the distal third of band 8q23 and the proximal two-thirds of sub-band 8q24.1 from normal GTG-banded chromosomes (Fig. 1). The dissected

region is estimated to comprise approximately 10,000 kilobases (kb). As the distal extent of the Langer-Giedion syndrome chromosome region is uncertain, we included chromosome material distal to the deletions in our two patients (see below and Fig. 3). To facilitate the identification and dissection of the chromosomes, the dissection was not performed in an oil chamber, and we used straight needles and pipettes, rather than microforged ones.

The dissected chromosome fragments were transferred to a collection drop kept in a moist chamber. After the dissection of 37 chromosomes, the microdrop was overlaid with oil. Chromosomal DNA (estimated yield, 0.5 pg) was digested with the restriction enzyme *RsaI* and ligated to a *SmaI*-cut pUC plasmid (Fig. 2). The inserts were then amplified by the polymerase chain reaction using the universal M13/pUC sequencing and reverse sequencing primers. In contrast to the amplification of specific DNA sequences in genomic DNA¹⁶, the universally primed polymerase chain reaction allows the simultaneous amplification of many different unknown DNA sequences. After 26 cycles of denaturation, annealing and DNA synthesis, the amplified inserts were released by *EcoRI* digestion and cloned into pUC13. We obtained 20,000 clones, of which we characterized 50 in detail.

The insert size ranged from 52 base pairs (bp) to 350 bp (mean, 150 bp). This is smaller than the mean size of *RsaI* restriction fragments (495 bp)¹⁷, probably because of the preferential amplification of small inserts. The cloning of small fragments has the advantage of yielding a high percentage of repeat-free clones: 10 inserts (20%) contained repetitive DNA and 40 inserts (80%) identified single-copy fragments. All but two of the single-copy clones were different. These clones were used to probe Southern blots containing DNA from two Langer-Giedion patients with a visible chromosome deletion.

Patient S.H. (ref. 18) has a deletion of band q23 and sub-band q24.11 (ref. 13); patient T.T. (ref. 19) has a deletion of the distal half of band q23 and the proximal half of sub-band q24.1 (ref. 13). Twenty clones (50%) map outside the deletions in S.H. and T.T. These clones are probably derived from the dissected material distal to the deletion in T.T., but cannot be assigned at present. Eleven clones are deleted in both patients, and nine clones are deleted in patient T.T. only (Fig. 3). These clones are currently being extended by chromosome walking and physically linked by pulsed-field gel electrophoresis. None of the microclones maps to the region deleted in S.H. only, which is proximal to the dissected region. This demonstrates the precision of our technique.

In addition to the region on chromosome 8, we have successfully dissected and cloned DNA from 11p13 (Wilms tumour-aniidria) and 15q11-12 (Prader-Willi syndrome). In these experiments, only 20 chromosomes were dissected and all of the clones tested so far map to the dissected bands. If desired,

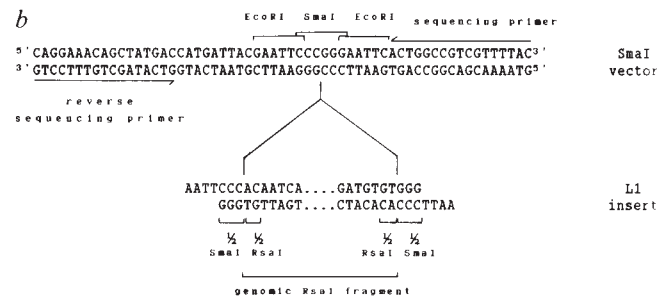
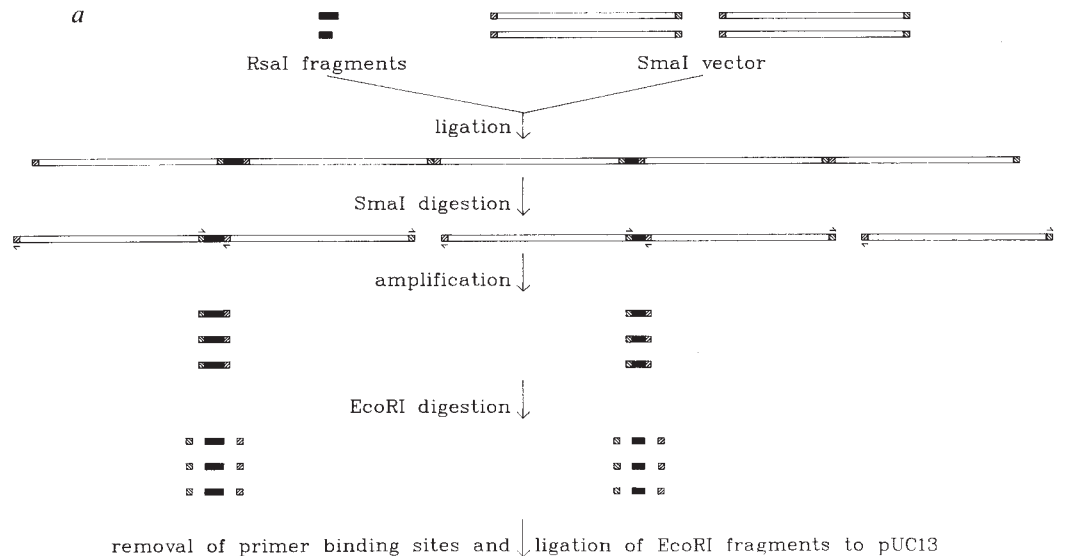
FIG. 1 Microdissection of chromosome 8. *a*, Human GTG-banded metaphase with two chromosomes 8, one dissected (8q23.3→8q24.21) and one not, for comparison. *b*, Diagram showing a normal G-banding pattern of a human chromosome 8 (ref. 20). The arrows on the left indicate the region dissected in all chromosomes 8. The arrows on the right mark the most proximal and most distal limits of the various cuts.

METHODS. Cells from normal amniotic fluid cell cultures were collected using the pipette method (time of fixation was 10–20 s)²¹. The chromosomes were spread onto clean coverslips, washed in 70% ethanol, air dried and GTG-banded. Microdissection was performed on an inverted microscope (IM Zeiss; magnification, 1,200 $\times$) with the aid of a micromanipulator (MR MOT; Zeiss) and extended siliconized glass needles. The dissected pieces ($n=37$) were pooled into a 1-ml collection drop (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 0.1% SDS, 1% glycerol, 500 $\mu\text{g ml}^{-1}$ proteinase K) which was situated in a small moist chamber on a separate siliconized coverslip. Proteinase K digestion and phenol extractions were performed under oil essentially as described⁹. The chromosomal DNA was digested with *RsaI* (Boehringer) at 37 °C for 2.5 h (final concentration 6 units μl^{-1}). Another aliquot of *RsaI* was added, and the mixture incubated for another 2.5 h. The enzyme was inactivated by three phenol extractions.



FIG. 2 Universal DNA amplification procedure. *a*, *RsaI* fragments (filled bars) are ligated to a fivefold molar excess of a *SmaI*-cut pUC vector (open bars) that contains a single *SmaI* site flanked by two *EcoRI* sites (see polylinker sequence in *b*). Polyethylene glycol is included in the ligation mixture to obtain long concatemers²². Nonrecombinant polylinker sequences are recut with *SmaI*, and inserts are amplified with the help of DNA polymerase and the universal M13/pUC sequencing (←) and reverse sequencing primer (→). After removal of the primer binding sites (hatched bars) by *EcoRI* digestion and gel filtration, the amplified fragments are cloned into pUC13. *b*, Polylinker sequence of the *SmaI* vector and insert sequence of clone L1. The *SmaI* vector is a pUC derivative with a single *SmaI* site flanked by *EcoRI* sites. It allows the ligation of *RsaI* fragments into the *SmaI* site and the release of the amplified inserts by *EcoRI* digestion. The released fragments are cloned into the *EcoRI* site of pUC13 (Pharmacia). As shown for one example (clone L1), the clone inserts contain half a *SmaI* and half a *RsaI* site at both ends.

METHODS. A synthetic *EcoRI*-*SmaI* adaptor (Pharmacia) was inserted between the two *EcoRI* sites of M13mp7 (BRL) to yield the symmetric polylinker sequence *EcoRI*-*SmaI*-*EcoRI*. The 320-bp *PvuII* fragment spanning the polylinker sequence of this M13 derivative was then used to replace the respective *PvuII* fragment of pUC19 to yield the *SmaI* vector. Chromosomal *RsaI* fragments (estimated yield 0.5 µg) were ligated to 13.5 µg *SmaI* vector in the presence of 15% polyethylene glycol (relative molecular mass 8,000). T4 DNA ligase (New England Biolabs; 0.08 units) was added, and the ligation was carried out at 15 °C overnight. The reaction mixture (128 nl) was then taken up into 2 µl of water and transferred to a microfuge tube. After heat inactivation of the ligase, non-recombinant polylinker sequences were recut with 2 units of *SmaI* (Boehringer) at 25 °C for 1 hour (final volume 5 µl). The sample was adjusted to 50 µl of 20 mM Tris-HCl pH 7.8, 5 mM MgCl₂, 1 mM dithiothreitol, 2.5 mM of each of the four deoxynucleoside triphosphates and 1 µM each of the M13/pUC sequencing and reverse sequencing primers. The DNA was denatured by incubation at 95 °C for 5 min, and the primers were allowed to anneal at 33 °C for 2 min. After the addition of 1 unit of Klenow DNA polymerase (MEDAC, Hamburg) in 1 µl amplification



buffer, DNA synthesis was carried out at 37 °C for 10 min. This cycle was repeated 25 times, except that subsequent denaturations were performed at 92 °C for 2 min, and the time for DNA synthesis was reduced to 5 min after the fifth cycle. After the last primer extension step, Klenow DNA polymerase was heat-inactivated and the amplification products were digested with *EcoRI*. The amplified fragments were purified by gel filtration through two successive Sephacryl S-200 spun columns (Pharmacia) and ligated to dephosphorylated *EcoRI*-cut pUC13 (Pharmacia). The ligation products were used to transform competent DH5α cells (BRL). Recombinant plasmids were purified on Qiagen-20 tips. DNA sequences were determined by the dideoxy chain-termination method²³.

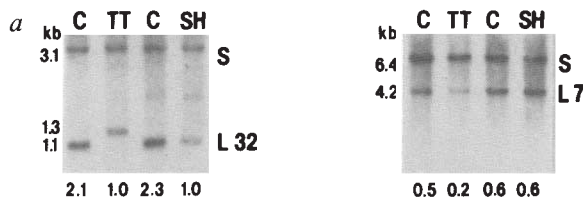
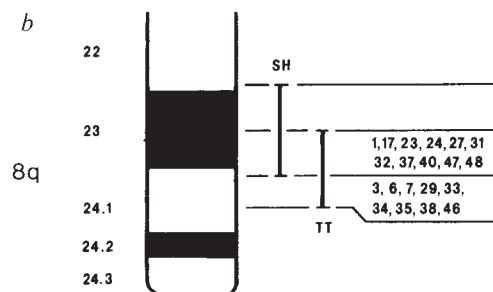


FIG. 3 Chromosomal localization of the microclones. *a*, Quantitative Southern blot analysis of Langer-Giedion syndrome patients. Genomic DNA from a normal control (C) and from the patients S.H. and T.T. was digested with *HindIII* and probed with the microclones indicated beside the bands. Hybridizations were performed essentially as described previously²⁴. Various probes that map outside the deletions served as an internal hybridization standard (S). Each lane contains approximately the same amount of DNA. The relative intensity of the autoradiographic signals L32:S and L7:S was determined using a Shimadzu CS9000 densitometer and is given beneath each lane. Clone L32 is deleted in S.H. and T.T., and clone L7 is deleted in T.T. only. Similar results were obtained in two other independent experiments. The



larger L32 band in T.T. represents a rare allele of a *HindIII* restriction fragment length polymorphism²⁵. *b*, All microclones were mapped by quantitative Southern blot hybridizations as shown above. In each case, three independent experiments were performed. The distal part of 8q and the deleted chromosome segments in S.H. and T.T. are shown. The numbers on the right refer to the clones found to be deleted in both patients and in T.T. only.

the number and the size of the dissected fragments could be reduced much further, but the number of amplification cycles would have to be increased. The universal DNA amplification system also allows the cloning of minute amounts of DNA from ancient tissue samples and the construction of random primed cDNA libraries from small cell numbers. □

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Isotype exclusion and transgene down-regulation in immunoglobulin- λ transgenic mice

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A GIVEN B lymphocyte makes an antibody containing either κ - or λ -light chains, but not both^{1,2}. This isotype exclusion is effected at the level of the rearrangement of the immunoglobulin gene segments^{3–5}, although by an unknown mechanism. An attractive possibility is that, following productive rearrangement of one of the light-chain loci, the newly synthesized light-chain polypeptide inhibits DNA rearrangement for the other isotype. To test such feedback regulation, we have created transgenic mice carrying a rearranged λ_1 -gene. By contrast with the B cells in normal newborn mice which are mainly $\kappa^+ \lambda^-$, the B cells in the newborn transgenic mice express λ - but not κ -chains. We propose that the synthesis of any light chain, be it κ or λ , that allows expression of IgM on the cell surface results in a cessation of all V-J joining. Interestingly, the limited light-chain repertoire of the transgenic mice does not persist and most adult B cells express endogenous κ -rearrangements and down-regulate the transgene.

Analysis of transgenic mice has provided strong evidence for a role for immunoglobulin polypeptides in mediating a feedback regulation of allelic exclusion^{6–10}. Thus, a transgenic κ -gene inhibits rearrangement of the endogenous κ -loci^{7,8}. It has not been established, however, whether the κ -transgene has a role in mediating isotype exclusion as, even in normal mice, rearrangement of the λ -loci is a rare event. We have therefore approached this problem by creating λ -transgenic mice to see whether the λ -transgene exerts an effect on rearrangement of the endogenous κ -loci. A plasmid was assembled that contains a rearranged mouse λ_1 -gene linked to a chimaeric $\alpha 2$ -heavy-chain gene (Fig. 1a). This $\alpha 2$ gene lacks the exons encoding the human membrane-anchoring sequences and the presence of this human $\alpha 2$ -gene allowed unambiguous identification of mouse cells expressing the transgene. Transcription of both the heavy- and light-chain genes is potentiated by the mouse IgH enhancer.

Linearized plasmid DNA was injected into mouse eggs. Of 42 mice born, 12 carried integrations of the injected DNA. Nine

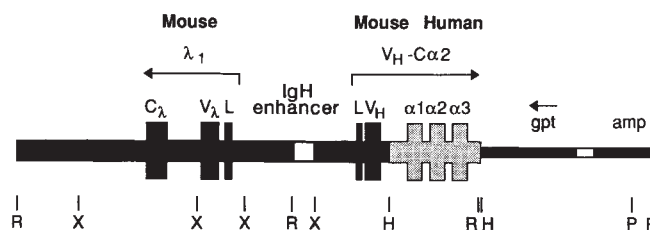


FIG. 1 Structure of plasmid DNA: thick filled lines, mouse immunoglobulin DNA; hatched lines, human immunoglobulin DNA; open boxes, IgH and SV40 enhancers; thin filled lines, pSV2gpt vector. Restriction sites are abbreviated: R, EcoRI; X, XbaI; H, HindIII; P, PvuI. The plasmid is a derivative of pSV-V_{NH}α2 described previously²¹ and contains a 7.4-kb EcoRI fragment including the rearranged λ_1 gene of the mouse HOPC2020 plasmacytoma²². Plasmid DNA was linearized at the PvuI site in the vector and transgenic mice were derived as previously described²³.

of these transgenic founder mice expressed the transgene as judged by enzyme-linked immunosorbent assays (ELISA) which detected the presence of IgA2, λ_1 anti-NP antibody in serum. Northern blot analysis (data not shown) revealed that expression of the transgene was lymphoid-specific. Most analyses were performed on descendants of founders 8,023 (which gave serum titres of $\sim 5 \mu\text{g ml}^{-1}$ at 4 weeks of age) or 8,032 (serum titres of $100\text{--}200 \mu\text{g ml}^{-1}$).

Analysis of cells from the offspring of founder 8,032 using the fluorescence-activated cell sorter (FACS) showed that the transgenic mice and their non-transgenic siblings contained similar proportions of IgM⁺ B cells in their spleens (Fig. 2a). The newborn transgenic mice, however, differ radically from their littermates with respect to immunoglobulin light-chain expression. The transgenics have few κ -bearing B cells, most carrying λ -chains on the surface (Fig. 2a). Cytoplasmic immunofluorescence analysis gave similar results (Table 1) and revealed that most of the λ^+ -cells stained for human $\alpha 2$, indicating that they were likely to be expressing the transgenic rather than an endogenous λ -gene. By contrast with the non-transgenic siblings, however, the isotype exclusion in the transgenic mice, although impressive, is far from absolute, with $\sim 10\%$ of the B cells making both κ - and λ -chains (Table 1). The $\alpha 2$ -heavy-chain itself was detected only intracytoplasmically.

Transgene expression among splenic B cells of adult transgenic mice contrasts strikingly with that in the neonates. The proportion of λ -expressing cells decreases considerably with age and there is a corresponding increase in the number of B cells that express κ -light chains (Fig. 2a and Table 1). This fall with age in the proportion of transgene-expressing cells is observed in F₁ and subsequent generation offspring of both the 8,023 and 8,032 founders. We believe that a small proportion

TABLE 1 Cytoplasmic immunofluorescence analysis of immunoglobulin light-chain expression

	Transgenic		Control	
	Neonates	Adults	Neonates	Adults
λ -only	73	25	17	3
κ , λ -doubles	10	9	0	0
κ -only	17	66	83	97

Results in each column show the percentage of total light-chain-positive spleen cells from pools of 3 mice of 8,032 offspring which type as κ -only, λ -only or κ , λ -doubles. Neonatal mice were 4 days old and adults 9 weeks old. Immunofluorescence on methanol-permeabilised cells was carried out using a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse λ -antiserum (Southern Biotechnology) and biotinylated goat anti-mouse κ -antiserum (Amersham) with phycoerythrin-conjugated streptavidin. It is notable that in the strain of mice used here (C57BL/6 $\times$ CBA F₁), the proportion of λ^+ cells in non-transgenic neonates is somewhat higher than that reported previously²⁵ for BALB/c and C57BL/6.

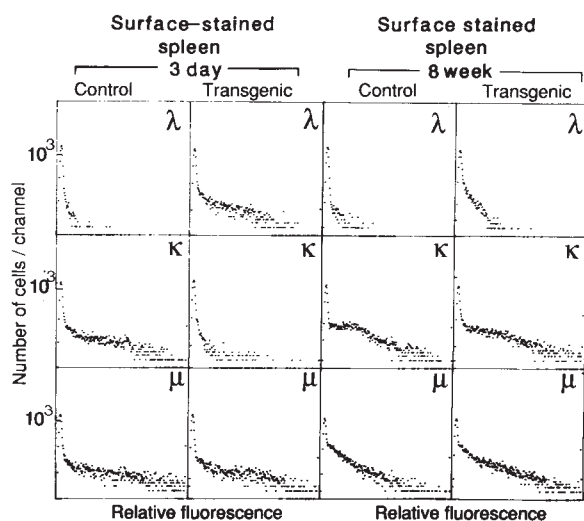


FIG. 2 Fluorescence analysis of transgene expression. FACS profiles of spleen cells surface-labelled with biotinylated sheep anti-mouse κ , λ or μ antibodies and fluorescein-conjugated streptavidin; dead cells were gated out by scatter gating. Samples were obtained from 3 day or 8-week-old offspring of 8,032. The results shown here are from four individual mice although similar profiles were obtained from other siblings using both neonatal and adult F_2 offspring of both 8,023 and 8,032.

of B cells either fails to activate or, alternatively, down-regulates expression of the transgene; these cells then undergo rearrangement of the endogenous light-chain loci, and selection at the cellular level results in the temporal change in antibody expression¹¹.

Hybridomas were established to ascertain whether the inhibition of κ -light-chain expression in the transgenic mice was due to an inhibition of κ -gene rearrangement. Spleen cells from 3.5- or 8-week-old transgenic mice were fused with the NS0 plasmacytoma. DNA was prepared from 22 randomly chosen cloned hybridomas that secreted λ -light chains. Of these 22 clones, three turned out to be κ , λ -double producers with the other 19 making λ as their sole light chain. Southern blot analysis (Fig. 3a) revealed that the κ -loci of spleen cell origin in the λ -only hybrids were always in the germline configuration, in stark contrast to those from λ -expressing B cells of non-transgenic

mice which normally bear aberrant κ -rearrangements on one or both alleles^{3-5,12}. Furthermore, the state of the κ -loci in the λ -hybridomas from the transgenic mice also contrasts with the κ -loci in random hybridomas made by fusing spleen cells from normal (CBA $\times$ C57BL/6) F_1 mice with NS0. In this case, DNA was analysed from randomly chosen, cloned hybridomas whether or not they produced immunoglobulin. Consistent with previous observations on primary cells¹³, we found that the incoming κ -gene was rearranged in 9 out of 20 of these hybrids (data not shown). These results show therefore that the presence of the IgA2, λ_1 transgene leads to an inhibition of κ -gene rearrangement.

The mechanism regulating the hierarchy of rearrangements at the light-chain loci remains a mystery. Apart from aberrant V_{κ} - J_{κ} joining, most λ -expressing hybridomas contain rearrangements involving the rearranging sequence element (RS)^{14,15}. This element, which in man is termed κ -deleting element (kde)^{16,17}, is located ~24 kilobase (kb) downstream of the C_{κ} exon and recombines with the joining signals belonging to a V_{κ} segment or in the vicinity of the J_{κ} elements with resultant destruction of the κ -locus. The significance of this RS recombination is not known. It has been proposed that it acts as a signal to initiate rearrangement at the λ -loci¹⁴. It is also possible, however, to envisage models in which productive λ -rearrangement triggers RS recombination and thereby ensures isotype exclusion. To test this second model, we looked at the status of the RS element in the λ -expressing hybridomas from the transgenic mice. Two main bands hybridizing with the RS probe are clearly visible (Fig. 3c); the strong 6-kb *Eco*RI fragment containing the germline RS element and a weak 13-kb band containing the rearranged RS of NS0. Several of the hybrids have lost the rearranged RS of the fusion partner, presumably through chromosome loss although we have no simple explanation for the fact that loss of the NS0, rearranged RS allele is particularly evident in the hybrids that express only λ -light chains. There is no evidence, however, of additional RS rearrangements in most of the hybridomas although one (NW473/22, a λ -only expresser, not shown) contains extra bands that hybridize weakly with the RS probe. On the basis of these results, it seems improbable that RS recombination is specifically activated by the presence of λ -light chains. Therefore, if RS recombination does indeed have a role in isotype exclusion it is more likely to lie in the activation of λ -rearrangement rather than in enabling λ -chains to switch off κ -rearrangement.

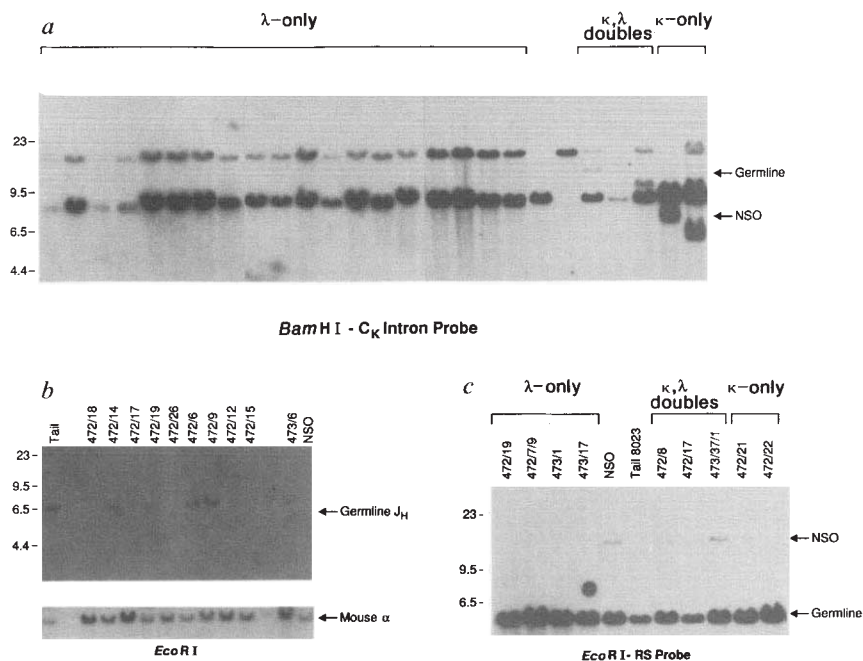


FIG. 3 Southern blot analysis of κ , IgH and RS rearrangements in splenic hybridomas. **a**, κ -rearrangements: DNA digested with *Bam*HI was probed with an *Xba*I-*Hind*III fragment from the mouse J_{κ} - C_{κ} intron. The bands labelled NS0 are from the NS0 fusion partner and Germline indicates the band corresponding to the germline, unrearranged κ locus. **b**, IgH rearrangements: DNA was digested with *Eco*RI and probed with a 0.6-kb *Sac*I-*Bsu*36I fragment from between D-Q52 and J_{κ} 1. The blot was then reprobed for the mouse immunoglobulin α -heavy-chain gene so as to provide a control for DNA loading. **c**, RS rearrangements: DNA was digested with *Eco*RI and probed with an 0.8-kb *Sau*3A fragment (probe rs0.8 of ref. 15) for RS sequences. N indicates the rearranged RS element of the NS0 fusion partner and G indicates the germline-configuration RS band.

METHODS. The hybrids were derived by fusing spleen cells from an 8-week-old offspring of 8,023 (NW472 hybrids) or a 3.5-week-old offspring of 8,032 (NW473 hybrids) with NS0 plasmacytoma cells. After growth in selective medium, hybrids were cloned by limiting dilution.

Apart from the inhibition of κ rearrangement, the transgenic mice also exhibit a decreased amount of rearrangement at the IgH loci. Of a total of 25 random λ^+ -hybrids examined from the transgenic mice, 13 (52%) contained a germline J_H allele as detected using a probe for the region located between D-Q52 and J_{H1} (one of the blots is shown in Fig. 3b). This is a considerably higher figure than would be expected from an analysis of IgH rearrangements both in primary B cells¹⁸ as well as in random hybrids generated by fusing spleen cells from (C57BL/6 $\times$ SJL) mice with X63.Ag8.653 (ref. 10). We cannot formally exclude the possibility that this inhibition is due to the secreted form of the human $\alpha 2$ heavy-chain polypeptide. A similar inhibition of IgH rearrangement, however, has recently been described in mice transgenic for a κ -light-chain gene where 22% of the hybrids were found to contain a germline J_H allele¹⁹. By analogy with the proposal of Manz *et al.*¹⁹, we suspect that the increased frequency of germline J_H loci in the hybridomas from the IgA2, $\lambda 1$ transgenic mice is probably caused by a combination of transgenic $\lambda 1$ chain with the endogenous μ_m -polypeptide leading to an early switch off of recombinase activity in pre-B-cells that have just rearranged their endogenous IgH locus. This could then lead to a diminished amount of D- J_H rearrangement on the excluded IgH allele. The fact that we see a higher proportion of germline J_H alleles in the IgA2, $\lambda 1$ transgenic mice than has been described in the κ -transgenics could be due to that fact that expression of the $\lambda 1$ transgene is potentiated by the IgH enhancer; it may therefore be switched on earlier than the κ transgene. Because of this inhibition of both heavy and light chain rearrangement, it is not surprising that the transgene causes an overall depression of B-cell development as the number of spleen cells in the transgenic mice was $\sim 70\%$ that of their non-transgenic siblings.

The analysis of immunoglobulin light-chain gene expression in mouse plasmacytomas and in hybridomas from mice transgenic for a κ -light chain^{7,8,12} supports a model in which V_K - J_K joining is switched off following a signal delivered by a complete IgM, κ antibody molecule. Recent data obtained using both transgenic mice and pre-B-cell lines were consistent with the membrane form of the IgM antibody mediating this feedback regulation^{9,20}. From the work described here it is clear that a transgenic λ -light chain (presumably in association with the endogenous μ_m -polypeptide) causes an inhibition of κ -locus rearrangement. It is probable that, following the production of intracellular μ_m polypeptide chains, any light-chain rearrangement be it λ or κ that leads to IgM on the cell surface results in a cessation of all V-J joining. It is attractive to speculate that whereas the presence of μ_m -polypeptide in the membrane of the endoplasmic reticulum signals a stop to heavy-chain rearrangement and a start to light-chain rearrangement, productive light-chain rearrangement causes the μ_m -polypeptide to be translocated to the plasma membrane where it signals to stop all V-gene rearrangement. $\square$

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A dominant control region from the human β -globin locus conferring integration site-independent gene expression

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THE regulatory elements that determine the expression pattern of a number of eukaryotic genes expressed specifically in certain tissues have been defined and studied in detail. In general, however, the expression conferred by these elements on genes reintroduced into the genomes of cell lines and transgenic animals has turned out to be at a low level relative to that of endogenous genes, and influenced by the chromosomal site of insertion of the exogenous construct. We have previously shown that if regions flanking the human β -globin locus are introduced into the mouse genome along with the human β -globin gene, a level of expression comparable to that of endogenous genes can be achieved that is also independent of integration site^{1,2}. We have now defined a dominant control region with these properties consisting of 6.5 kilobases of DNA encompassing erythroid cell-specific DNase I hypersensitive sites. The identification of such dominant control regions could have important applications in somatic gene therapy.

The human β -globin 'minilocus' construct contained 21 kilobases (kb) of DNA from the region 5 to the ϵ -globin gene encompassing four erythroid cell-specific DNase I hypersensitive sites and 12 kb of DNA 3' to the human β -globin locus¹. To investigate the properties of these dominant control region (DCR) sequences, the 33 kb 5' and 3' sequences were replaced by a 6.5 kb DNA fragment containing only the upstream hypersensitive sites^{1,3,4} (Fig. 1). Construct 1359 contains the four hypersensitive sites in the same orientation relative to the β -globin gene (and the *tk-neo*^r gene; tk, thymidine kinase; neo^r, neomycin-resistance) as that found on chromosome 11. Construct 1400 has the DNase I hypersensitive sites in the opposite orientation and 1401 and 1357 have the four DNase I hypersensitive sites placed 3' to the β -globin gene (Fig. 1).

The insert of construct 1359 was injected into fertilized mouse eggs and 56 embryos were collected after 13.5 days of gestation. Thirteen transgenics were obtained in two groups as defined by S1 nuclease protection (Fig. 2a) and Southern blot analysis to measure the expression levels and copy number of the human β -globin gene in the fetal liver (Table 1). Members of the first group expressed the insert at low levels because they were mosaics (three mice, for example lane 2), or carried a deletion in the insert (two mice, for example lane 3). The remainder express the gene at high levels in a copy-number dependent way: five of these looked normal (for example lane 4) and three looked anaemic (for example lane 1). These results are therefore similar to those obtained with the minilocus¹ and show that full erythroid-specific activation is obtained by the small reconstructed DCR, sometimes leading to a thalassaemia-like anaemia.

To study the different β -globin constructs and avoid mosaicism, three independent, stably transformed, MEL cell popula-

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tions were generated for each construct¹. RNA was prepared before (-) or after (+) differentiation⁵ and analysed on northern blots with probes of similar specific activity. Figure 2b shows that human β -globin mRNA is present at levels as high as those of endogenous mouse globin mRNA after induction. For comparison, Fig. 2b shows equal amounts of RNA from two populations using a construct (1273) without the DCR. Human β -globin expression is increased at least 100-fold by the addition of the four DNaseI hypersensitive sites and this high level of expression is observed in all orientations and relative positions (1359, 1400, 1401 and 1357). Moreover, this level of expression seems to be higher than that seen previously with the β -globin minilocus² (1016, Fig. 2b). We used S1 nuclease analysis to quantitate the levels of mRNA using probes for the human β , mouse α and mouse β^{maj} globin genes (Fig. 3a and Table 1). Hybridization signals from Southern blots to probes for *tk-neo*^r and human β -globin genes were compared to those from the endogenous single-copy mouse Thy-1 gene and average β -globin gene copy numbers were calculated (Fig. 3b, Table 1). The expression per gene copy ratio is 120% per human β -globin gene compared to the endogenous mouse β^{maj} -globin gene (see Table 1 legend). As observed in the embryos, the expression levels seem to be directly proportional to the gene copy number

as was observed for the human β -globin minilocus^{1,2}, although this argument is weakened by the fact that all the MEL populations generated have a similar average gene copy number (Fig. 3b, Table 1).

Figure 2b also shows that the 6.5 kb DNA fragment activates the heterologous herpes simplex virus (HSV) *tk* promoter in a similar way (compare 1359 to 1273). Unlike β -globin expression however, *neo*^r transcripts are present at about the same level as with the β -minilocus cosmid (ref. 2 and 1016), possibly because the *tk-neo*^r in the minilocus may already be transcribed maximally. Another difference between the minilocus and plasmid constructs is the higher level of β -globin and the *tk-neo*^r transcripts before erythroid induction (Fig. 2b and ref. 2). This may be due to the loss of specific sequences or to the greatly reduced size of the constructs. Therefore, the reconstructed DCR does not isolate the β -globin gene from the effects of flanking sequences before differentiation.

To investigate further the activation of the heterologous HSV *tk* promoter, constructs were prepared which carried the 6.5 kb DCR without any human β -globin gene sequence (1417) or carrying the previously described enhancers, either from within (1139) or from the 3' flanking region of (1138) the β -globin gene^{6,7,8,9}. Activation and erythroid inducibility of the HSV *tk* promoter is not dependent on the promoter or enhancers of the β -globin gene (Fig. 2c).

Interestingly the human β -globin gene expression per gene copy is increased, when compared to the β -globin minilocus (1016, Fig. 2b), but the *tk-neo*^r expression remains the same. The transcription machinery of the *tk* promoter may have been

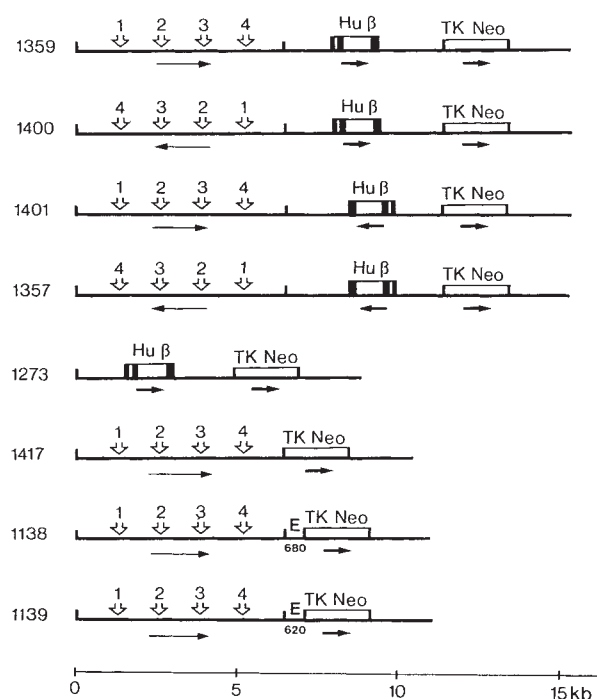


FIG. 1 Construction of the human β -globin plasmid locus.

METHODS. Plasmids 1359, 1400, 1401 and 1357 were constructed using restriction endonuclease fragments encompassing the 5' DNaseI hypersensitive sites ligated into a pPoly-III-I vector (a gift from A. J. Clark, Edinburgh) in the same orientation as in the normal β -globin locus: 2.1 kb *Bam*HI-*Xba*I fragment containing hypersensitive site 1, a 1.9 kb *Hind*III fragment containing hypersensitive site 2, a 1.5 kb *Asp*718-*Bgl*II fragment containing hypersensitive site 3 and a 1.0 kb partial *Sst*I-*Hind*III fragment containing hypersensitive site 4. A 6.5 kb *Not*I fragment containing all of the restriction fragments of the hypersensitive sites was then cloned into a Bluescript vector (1273) containing the 4.8 kb *Bgl*II fragment of the human β -globin gene¹ and a 2.7 kb partial *Eco*RI *tk-neo*^r gene fragment from the cosmid pTCF (ref. 15). Vertical arrows show arrangement of the hypersensitive sites in the dominant control regions but do not show their actual positions within these constructs. Construct 1417 contains the 6.5 kb *Not*I fragment containing the four 5' hypersensitive sites cloned into a specially designed polylinker in a pUC18 vector containing the 2.0 kb partial *Nar*I-*tk-neo*^r gene fragment. Construct 1138 contains the 680 bp *Dra*I-*Acc*I fragment of the 3' human β -globin enhancer cloned between the *Kpn*I and *Clal* sites of 1417. Construct 1139 contains the 620 bp *Dra*I fragment of the internal enhancer of the human β -globin gene⁹. Construct 1016 is the β -globin minilocus cosmid^{1,2}.

TABLE 1 Copy number and expression levels of the human β -globin gene in transgenic mice and transfected cells

		c.p.m. Hu β	Copy number Hu β -globin	Percentage expression		
				Hu β / Ma	Hu β / M β^{maj}	Ma/ M β^{maj}
Mouse 1		315*	4.0	ND	160	ND
	2	449	mosaic	ND	—	ND
	3	654	deletion	ND	—	ND
	4	5,385	3.0	ND	160	ND
1357	a	14,738	7.0	70	130	190
	b	10,859	4.3	90	130	140
	c	10,692	2.6	110	210	200
1359	a	12,692	7.7	70	110	170
	b	18,855	7.0	100	140	150
	c	17,533	7.0	80	150	200
1400	a	19,670	5.8	90	100	100
	b	20,924	5.8	90	90	100
	c	18,695	6.2	90	90	100
1401	a	14,643	3.4	120	120	100
	b	17,835	4.5	100	110	110
	c	13,262	4.9	70	70	100
Average expression/gene copy				90	120	140
Standard deviation				16	36	42
1359	a	39,709	7.7	60	120	180
	3x b	55,012	7.0	90	150	160
	c	56,059	7.0	80	160	210
1016	a	2,684	5.0	18	50	270
	h	1,289	7.0	11	30	280

Copy numbers of human β -globin (Hu β) were determined by Southern blotting and laser densitometry as described. Background-corrected Cerenkov counts for human β -globin, mouse β -globin major and mouse α -globin protected fragments were expressed as ratios of the exogenous human β -globin message (Hu β) to the endogenous mouse α and β^{maj} signals (Ma and M β^{maj}), correcting for relative specific activities. This expression ratio was then reduced to an expression per gene copy ratio by dividing it by the ratio of the number of copies of human β -globin to the number of endogenous gene copies (two for mouse β -globin major and four for mouse α -globin). Clones a and h seem to have threefold lower human β -globin expression than the average minilocus MEL population due to the loss of endogenous mouse β -globin genes². ND, not determined, * Anaemic.

saturated, which also explains why the addition of human β -globin gene enhancers to the 6.5 kb DNA fragment does not result in any further increase in the number of *tk-neo*^r transcripts in MEL cell populations (Fig. 2c). We therefore suggest that a requirement for reproducible but low expression levels of biologically active molecules could be met by the combination of the DCR and inefficient or mutagenized promoters.

DNaseI fadeout analysis on nuclei from uninduced cells

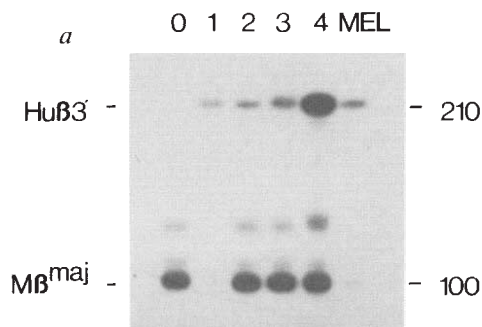
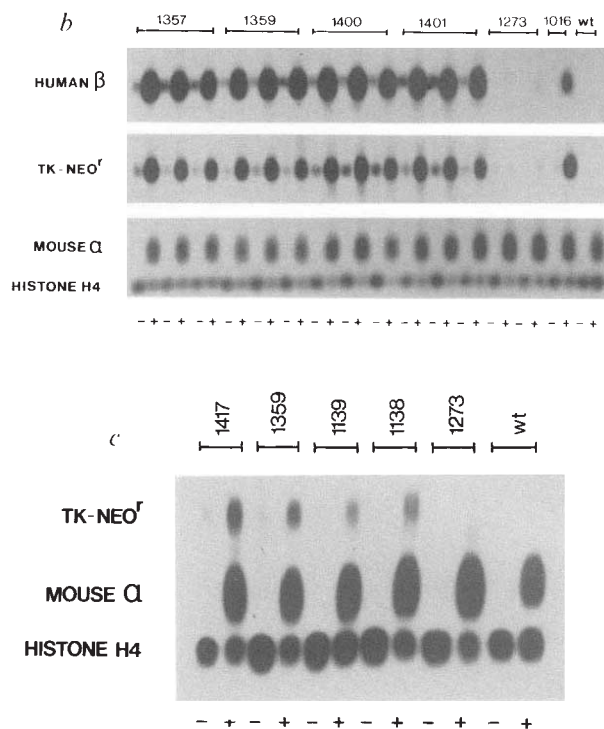


FIG. 2 Analysis of human β -globin expression. *a*, S1 nuclease protection analysis of RNA from the transgenics (sizes are shown on the right). *b*, Northern blot of RNA prepared before (–) or after (+) differentiation of the stably transformed MEL cell lines. Lanes marked 1016 contain RNA from a MEL cell clone stably transfected with the human β -globin minilocus². Wt, RNA from untransfected MEL C88 cells. The probes were for human β -globin (760 bp *EcoRI*–*MspI*) or the *tk-neo*^r gene (580 bp *SphI*–*BglII*). The filter initially probed with human β -globin was reprobed with both the mouse histone H4 probe (460 bp *EcoRI*–*Tth1111*) and the mouse α -globin probe (300 bp *BamHI*). *c*, Northern blot of total RNA (10 μ g) from uninduced (–) and induced (+) transfected C88 cell populations, probed simultaneously with the *tk-neo*^r probe, the mouse α -globin probe and the mouse histone H4 probe.

METHODS. In (*a*), an 11.5 kb *EcoRV* fragment from construct 1359 was gel-purified and microinjected into fertilized mouse eggs¹. The levels of human β -globin mRNA were determined by S1 nuclease analysis with a mixture of probes for the human β -globin and mouse β ^{maj}-globin genes^{1,2}. After autoradiography, each band was excised from the gel, placed at the bottom of an Eppendorf tube and quantitated by Cerenkov counting. A local background count was obtained by measuring a gel slice immediately above the band of interest and was subtracted from the actual count in subsequent calculations (see Table 1). For the Northern blots (*b*, *c*), plasmid DNA of indicated constructs (100 μ g) was linearized by digestion with *PvuI* and introduced into MEL C88 cells by electroporation⁹. Three independent G418-

shows that the hypersensitive sites 1–4 are regenerated in uninduced MEL cells, but not in L-cells with the exception of site 3 (data not shown and ref. 2). Weaker DNaseI hypersensitive sites can be seen on the β -globin gene promoter, 3' enhancer and *tk-neo*^r promoter. Therefore, at this level of resolution, the establishment of an 'active' chromatin configuration is separable from full β -globin gene transcription.

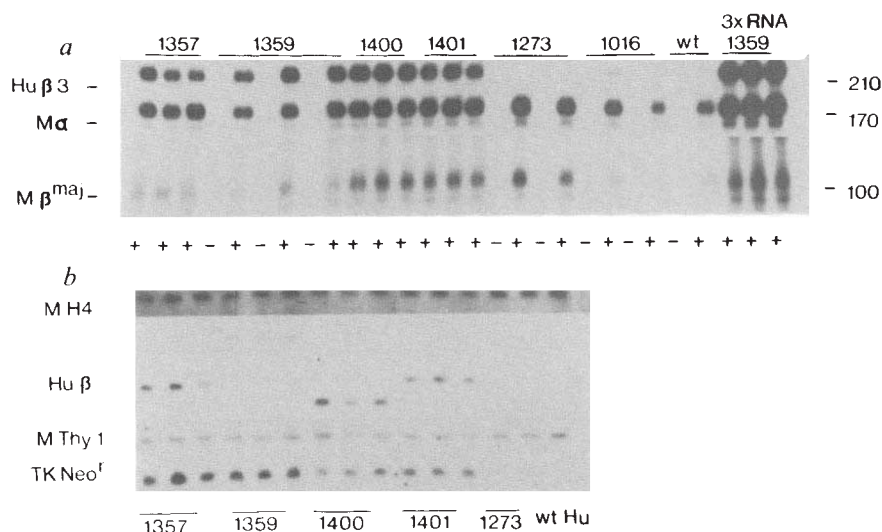
The human β -globin gene constructs in these experiments are



resistant populations of 50–100 clones were generated for each construct. Total RNA (10 μ g) isolated by LiCl/urea extraction¹⁶ from uninduced MEL cells, and MEL cells induced to differentiate by the addition of 2% v/v dimethyl sulphoxide (+) for five days, was electrophoresed on duplicate 1.5% agarose denaturing gels containing 0.7% formaldehyde and ethidium bromide (1 μ g ml^{–1}) blotted to nitrocellulose and probed.

FIG. 3 S1 nuclease analysis and copy number determinations. *a*, S1 nuclease analysis of total RNA from uninduced (–) and induced (+) MEL cells. *b*, Southern blotting of genomic DNA from MEL populations.

METHODS. (*a*) Total RNA (10 μ g) from uninduced (–) and induced (+) populations of MEL cells were hybridized to a mixture of mouse β -globin major, mouse α -globin and human β -globin probes described previously^{1,2}. Relative specific activities were 1, 10 and 8, respectively, for the above probes. Three controls containing 30 μ g of RNA (3 $\times$ RNA) were performed to prove that the S1 probes were in excess. Quantitation was as described in Fig. 2a. 1016 RNAs are from two MEL cell clones containing the human β -globin minilocus². Sizes are shown on the right. *b*, Genomic DNA (8 μ g) of MEL populations was digested with *EcoRI* and electrophoresed on a 0.6% agarose gel. After Southern blotting, the filter was probed with human β -globin intervening sequence probe (900 bp *BamHI*–*EcoRI*) and subsequently with a *tk-neo*^r resistance probe (580 bp *SphI*–*BglII*), a mouse Thy-1 probe (M Thy 1) (600 bp *PstI*) and a histone H4 probe (M H4) (460 bp *EcoRI*–*Tth1111*). Laser densitometry using a range of autoradiographic exposures was used to quantitate human β -globin copy number. Mouse Thy-1 and histone H4 were used to correct for DNA loading. Human placental DNA was used to obtain an estimate of the actual β -globin copy



number. The human β -globin copy number of the populations of cells containing 1359 were compared to the other constructs through the *tk-neo*^r gene signal as the *EcoRI* digestion gave a larger 9.3 kb human β -globin *EcoRI* fragment.

integrated at different sites into host cell DNA and have been replicated as chromatin. This contrasts with transient transfections using extra-chromosomal DNA templates not packaged into chromatin. Viral enhancers can stimulate β -globin gene expression several hundredfold in transient assays¹⁰, but this is not observed after stable integration, and levels of expression are dependent upon the site of integration into host DNA.

The observation that only 6.5 kb of DNA from the 5' boundary of the human β -globin locus allows high levels of expression of the human β -globin gene and the heterologous *tk-neo*^r gene in erythroid cells has implications for the prospects of somatic gene therapy. Currently, the most realistic approach for therapy of a β -globin gene disorder (thalassaemia or sickle-cell anaemia) is by *in vitro* retroviral infection of bone marrow and transplantation¹¹⁻¹⁴. Retroviruses carrying the human β -globin gene give rise to low levels of expression that are dependent upon the site of integration. The observation that the DCR works equally efficiently in all orientations and positions should facilitate production of retrovirus stocks with higher titres. The size of the reconstituted DCR with a smaller human β -globin gene

is within the range that can be packaged into retroviral particles. Such constructs have been made and are currently being tested. □

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Identification of globular mechanochemical heads of kinesin

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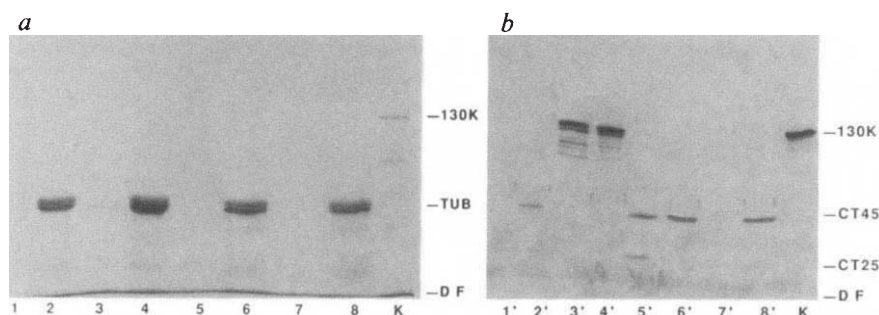
KINESIN is a mechanoenzyme which uses energy liberated from ATP hydrolysis to transport particles towards the 'plus ends' of microtubules¹⁻⁶. The enzyme consists of two polypeptide heavy chains of relative molecular mass (M_r) $\approx$ 110,000-140,000 (110K-140K) plus copurifying light chains; these polypeptides are arranged in a structure consisting of two globular heads attached to a fibrous stalk which terminates in a 'feathered' tail⁷⁻¹¹. Here we report that a function-disrupting monoclonal antikinesin, which binds to the 45K fragment of the kinesin heavy chain^{12,13}, recognizes an epitope located towards the N-terminal end of the heavy chain, and decorates the two globular heads lying at one end of the intact molecules (one antibody per head). The results show

that the two heavy chains of native kinesin are arranged in parallel, and that the 45K fragments, which display nucleotide-sensitive interactions with microtubules^{12,13}, represent mechanochemical 'heads' located at the N-terminal regions of the heavy chains. Thus, it is likely that the kinesin heads are analogous to the subfragment-1 domains of myosin.

Electron microscopy^{7,10,11} and DNA sequence analysis¹⁵ indicate that kinesin heavy chains are organized into distinct domains. We have obtained evidence for such domains from the proteolytic cleavage of kinesin, which yields heavy-chain fragments of $M_r \approx$ 45K and 76K^{12,13}. The 45K fragment is bound by three monoclonal antikinesins that inhibit kinesin-driven microtubule motility¹², one of which, SUK 4, was used in the present study. Interestingly, the proteolysis reaction that produces the SUK 4-reactive 45K fragments is nucleotide- and microtubule-sensitive (Fig. 1). Irrespective of the presence or absence of microtubules, the 130K kinesin heavy chain is resistant to cleavage in the absence of nucleotide whereas the formation of the 45K peptide is enhanced by magnesium ATP. In the absence of microtubules, MgATP also promotes the formation of a small quantity of a 25K subfragment of the 45K fragment (Fig. 1, lane 5). The presence of AMPPNP (an unhydrolysable ATP analogue) or ADP seems to favour subdigestion of the 45K domain in the absence of microtubules; consequently no SUK 4-reactive products are visible on immunoblots. The 45K fragment is, however, stabilized by the presence of microtubules in AMPPNP or ADP. It seems therefore that the proteolytic reactions that produce and subdigest the 45K fragment are affected by the conformational differences of kinesin-micro-

FIG. 1 Nucleotide and microtubule-sensitive proteolysis of sea-urchin kinesin 130K heavy chains. *a*, Coomassie-stained SDS polyacrylamide gel; *b*, corresponding immunoblot probed with the function-blocking monoclonal antikinesin SUK 4, which binds to the 45K peptide¹².

METHODS. Partially purified sea-urchin kinesin (lanes marked k) was obtained by AMPPNP-microtubule affinity binding, MgATP release, and Biogel A5M chromatography in the absence of MgATP (ref. 12). The kinesin in our standard PMEG buffer⁵ was digested with α -chymotrypsin¹² in the presence (lanes 2, 4, 6, 8) or absence (lanes 1, 3, 5, 7) of microtubules (2 mg ml⁻¹) assembled using taxol from purified bovine brain tubulin. Lanes 3 and 4 were incubated with apyrase 50 μ g ml⁻¹ to remove residual ATP or ADP; lanes 5 and 6 show digests performed in MgATP (5 mM), whereas 7 and 8 were performed in the presence of Mg AMPPNP (5 mM). Lanes 1 and 2 show digests performed using kinesin from the Biogel column and which presumably contained bound MgADP (ref.



4) (identical results were obtained in parallel digestion reactions performed in the presence of additional MgATP (1 mM) although in the presence of NaCl (0.5 M) microtubules did not protect the 45K peptide). 130K, intact kinesin heavy chain; TUB, tubulin; CT45, the 45K chymotryptic fragment of the kinesin heavy chain; CT25, a minor 25K fragment; DF, dye front.

tubule-nucleotide states and thus resemble the proteolysis of the myosin-head domain, a reaction which is affected by the molecular movements that occur as myosin interacts with nucleotides and actin¹⁶. We propose therefore that the 45K fragment is a mechanochemical domain on kinesin that contains nucleotide- and microtubule-binding sites^{12,13}, and undergoes conformational changes as it interacts with microtubules and nucleotides to generate motile force.

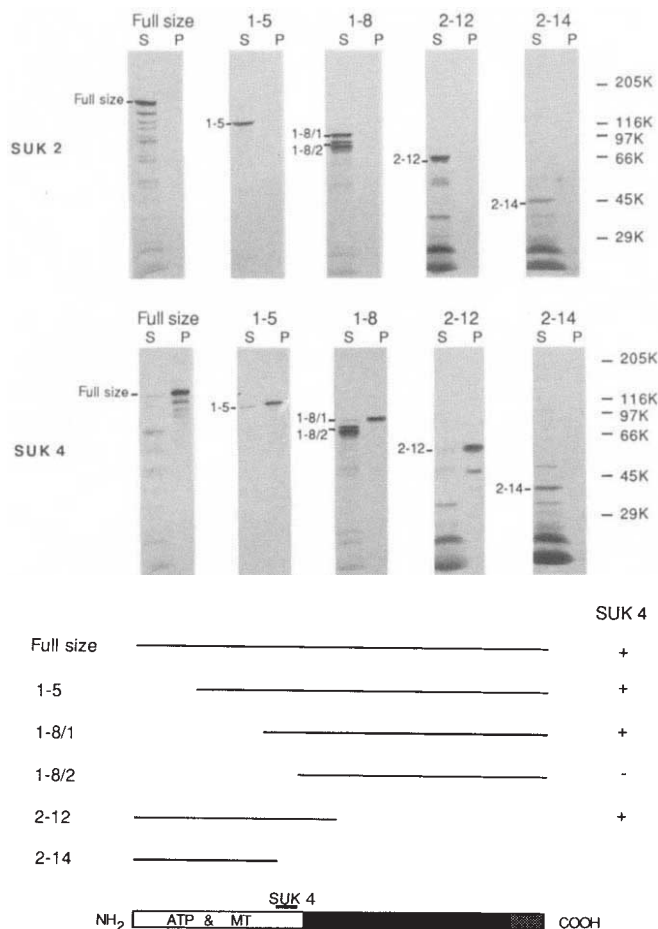


FIG. 2 Localization of the region of kinesin containing the SUK 4 epitope by immunoprecipitations of truncated *Drosophila* kinesin heavy-chain molecules. *a*, Upper panels, autoradiograms of the immunoprecipitation by SUK 2 and SUK 4. Each polypeptide is indicated by a line and its number in the corresponding autoradiogram. S, supernatant; P, pellet. *b*, Schematic representation of the kinesin heavy chain showing the position of the SUK 4 epitope, and the globular N-terminal domain (open box) and the nucleotide (ATP) and microtubule (MT) binding regions defined by previous studies¹⁵. Translation products that reacted with SUK 4, +. Black region, the α -helical coiled-coil domain, which ends in a second globular domain (shaded) at the terminus. In this model, SUK 4 lies in residues 320–390 and is near a microtubule binding region.

METHODS. Deletions were introduced into the full-length *Drosophila* kinesin heavy-chain cDNA, cloned into pBluescript plasmids (Stratagene) as described previously^{14,15} and the truncated products were transcribed, and translated using a reticulocyte lysate system^{14,15}. Six translation products were analysed: the full size *Drosophila* kinesin heavy-chain polypeptide consisting of 975 residues; 1-5, which lacks the first 130–170 N-terminal residues; 1-8/1, which lacks ~320 N-terminal residues; 1-8/2, which arises as a result of an internal initiation even in the same cDNA that produces 1-8/1 (ref. 15), and lacks ~390 N-terminal residues; 2-12, which lacks 500 C-terminal residues and 2-14 which lacks 640 C-terminal residues. The lysates containing the expressed, truncated products were incubated with monoclonal antikinesin, SUK 4, which cross-reacts with *Drosophila* kinesin heavy chains¹², and (as a control) with SUK 2, which reacts with sea urchin but not *Drosophila* kinesin heavy chains¹². The antibody-antigen complexes were immunoprecipitated using pansorbin *Staphylococcus aureus* cells, and subjected to SDS gel electrophoresis and autoradiography to locate the precipitated polypeptides.

To locate the 45K fragment on the kinesin heavy chain, we first determined the position of the SUK 4 epitope in the protein sequence (Fig. 2). Kinesin heavy-chain molecules carrying deletions of various lengths were produced by expressing truncated versions of the *Drosophila* kinesin heavy-chain gene^{14,15}. The binding of SUK 4 to the truncated protein products was assayed by immunoprecipitation: SUK 2, an IgG1 that does not bind to *Drosophila* kinesin¹², was used as a negative control.

Six translation products were analysed (described fully in ref. 15 and briefly in Fig. 2). As expected, SUK 2 did not immunoprecipitate any of the translation products. The full-size polypeptide (975 residues) and the truncated proteins 1-5, 1-8/1 and 2-12 but not 1-8/2 or 2-14, however, were immunoprecipitated by SUK 4. Significantly, molecules carrying deletions that include residues ~320–390 were not immunoprecipitated by SUK 4. These results suggest therefore that the epitope recognized by SUK 4 lies within the N-terminal third of the kinesin heavy chain, and is probably within residues 320–390. Strikingly, previous studies by Yang *et al.*¹⁵ show that the truncated kinesin heavy chain, 1-8/1, binds to microtubules weakly in sedimentation assays, whereas 1-8/2 does not bind microtubules at all. This indicates that the SUK 4 epitope is located near a region of the kinesin heavy chain that interacts with microtubules.

Yang *et al.*¹⁵ determined the sequence of the *Drosophila* kinesin heavy chain, and predicted that it consists of three structural domains: a globular N-terminal domain; a central α -helical coiled coil region; and another globular domain at the C-terminal region. In their proposed model, the N-terminal globular domain contains nucleotide- and microtubule-binding sites¹⁵ as well as the SUK 4 epitope (Fig. 2). The SUK 4 epitope and the microtubule- and nucleotide-binding sites are also all localized on the 45K fragment of kinesin, indicating that this 45K peptide corresponds to the globular N-terminal domain predicted from the sequence analysis.

To test this interpretation, we used immunoelectron microscopy to visualize the region of kinesin containing the SUK 4 epitope. The lower panels of Fig. 3*a* show freeze-etch electron micrographs of individual kinesin molecules as elongated bipolar structures approximately 75 nm in length. Each has two small, symmetrical globular 'heads' (9–10 nm in diameter) at one end of a long stalk (3–5 nm in width), which terminates in an irregular, bipartite 'feathered tail'. The appearance of this tail varies among molecules more than any other ultrastructural feature. One interpretation of these images is shown in Fig. 3*b*. The upper panels in Fig. 3*a* show electron micrographs of kinesin molecules decorated with SUK 4. This antibody clearly binds to the globular heads of the molecule, resulting in a marked increase in mass. Typically, this mass is the size of two IgG molecules (diameter 30 nm) and consists of at least six smaller lobes. Because IgG molecules themselves have a tri-lobed appearance¹⁷, we conclude that each of the two heads binds its own SUK 4 antibody.

Our results indicate that the N-terminal regions of two heavy chains form globular heads on the kinesin molecule (Fig. 3*b*). We propose that these heads are 'motor' domains undergoing changes in conformation as they interact with nucleotides and microtubules to generate force and motion, and that antibodies such as SUK 4 which bind to these heads can inhibit kinesin-driven motility by interfering with mechanochemical coupling. The junction between the head and the stalk may be flexible, rendering it highly susceptible to proteolysis, as in the case of myosin. In this model, the 45K fragments correspond to detached globular heads that are analogous to myosin subfragment 1(S1). The microtubule-induced ATPase activity of the 45K fragment, like the actin-induced ATPase activity of myosin S1 (ref. 18), is higher than that of the parent enzyme from which it is derived¹³, but whether the mechanism responsible for the elevation of ATPase activity is the same in these two cases remains to be determined.

Kinesin, myosin and dynein thus show striking similarities

a

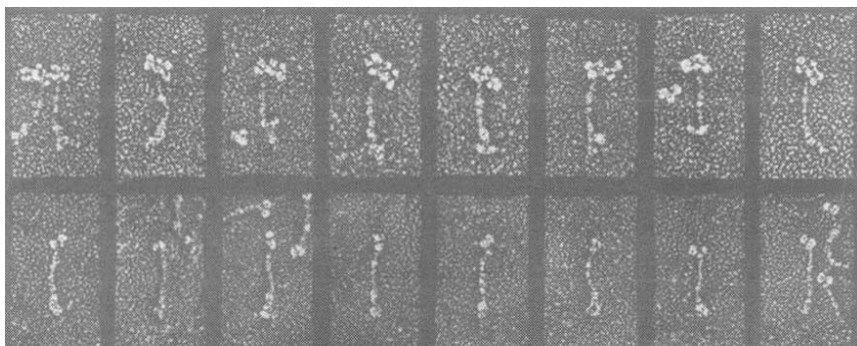
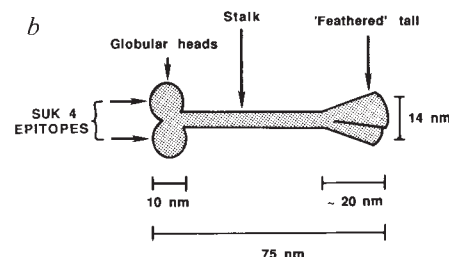


FIG. 3 *a*, Electron micrographs comparing individual sea-urchin kinesin molecules (lower row) with kinesin molecules reacted with the monoclonal antikinesin SUK 4 (upper row). The antibody binds to the two globular heads at the upper ends of the molecules. These represent rotary-shadowed platinum replicas of kinesin molecules (prepared as in Fig. 1 in PMEG buffer without nucleotide) that were adsorbed to microscopic flakes of mica and then freeze-dried by techniques described previously¹⁷. Magnification 144,000 $\times$. *b*, Model of the ultrastructural morphology of individual kinesin molecules. The globular heads, corresponding to the N-terminal domains predicted from the amino-acid sequence¹⁵, contain the ATP and MT binding sites, and the SUK 4 epitopes. The stalk is thought to consist of an α -helical coiled coil region (designated by the black area in Fig. 2), which terminates in a heterogeneous, bipartite feathered tail (the shaded C-terminal region in Fig. 2 is thought to interact with additional material to generate structures of the size seen in the micrographs¹⁵).

in their morphology, consisting of multiple globular mechanochemical heads attached to flexible stalks. The significance of this structural organization, however, is not clearly understood. It is possible that by having more than one head the mechanoenzyme remains attached as it tracks along its cytoskeletal filament with each head undergoing cycles of attachment and detachment. Single-headed myosin-1, however, also seems able to generate sliding between adjacent actin filaments²⁰, and to transport beads and organelles along cytoskeletal filaments^{21,22}. Johnson²³ has suggested that the flexible segment allows the production of a unidirectional force by mechanoenzymes whose motor domain attaches to its filament and undergoes equal and opposite structural changes during two different steps within the mechanochemical pathway. □

Note added in proof: Hirokawa *et al.*²⁴ have independently arrived at the conclusion that the two small heads on the kinesin molecule bind to microtubules.



High-resolution (1.5 Å) crystal structure of phospholipase C from *Bacillus cereus*

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BOTH the phosphatidylinositol-hydrolysing and the phosphatidylcholine-hydrolysing phospholipases C have been implicated in the generation of second messengers in mammalian cells^{1,2}. The phosphatidylcholine-hydrolysing phospholipase C (PLC) from *Bacillus cereus*, a monomeric protein containing 245 amino-acid residues³, is similar to some of the corresponding mammalian proteins⁴. This, together with the fact that the bacterial enzyme can mimic the action of mammalian PLC in causing, for example, enhanced prostaglandin biosynthesis⁵, suggests that *B. cereus* PLC can be used as a model for the hitherto poorly characterized mammalian PLCs. We report here the three-dimensional structure of *B. cereus* PLC at 1.5 Å resolution. The enzyme is an all-helix protein belonging to a novel structural class and contains, at least in the crystalline state, three Zn²⁺ in the active site. We also present preliminary results from a study at 1.9 Å resolution of the complex between PLC and inorganic phosphate (P_i) which indicate that the substrate binds directly to the metal ions.

The crystal structure of PLC has been solved at 2.8 Å resolution using multiple isomorphous replacement and solvent flattening⁶, and refined to 1.5 Å through the intermediate resolutions at 2.5, 2.3 and 1.9 Å. The present *R*-factor, using all the data and including 235 water molecules, is 15.7%. The molecular structure is shown in Figs 1 and 2. The crystallographic work, summarized in Table 1, will be published in more detail elsewhere.

The most striking feature of the molecule is the long anti-parallel helix pair formed by helix H and helices F and G, which can be compared with those of citrate synthase⁶ and a short helix pair in phospholipases A₂ (ref. 7). Proline 218 in helix H induces a bend between helices H₁ and H₂. Helix E lies across both this bend and the loop between helices F and G, giving the impression that the long helices are folded round it. The bend in helix B at Trp 43 is probably associated with its packing against the body of the molecule.

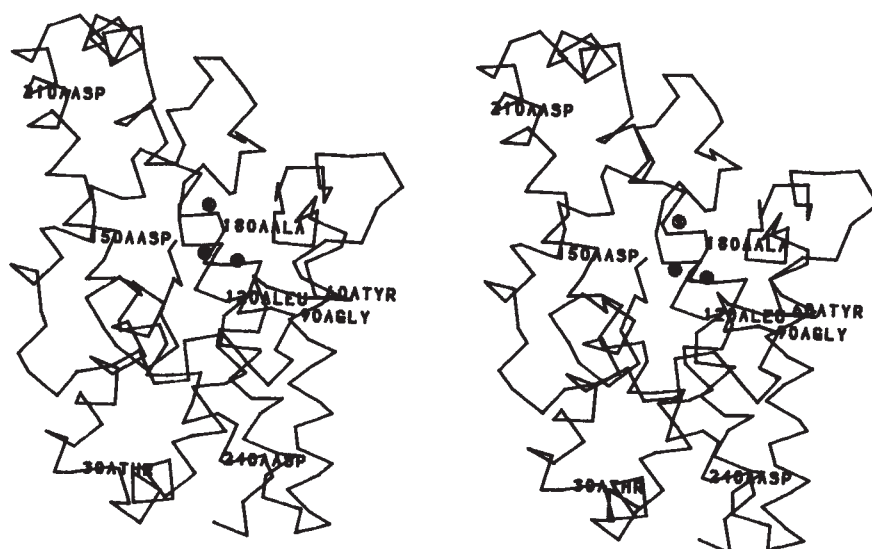
‡ Deceased.

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FIG. 1 Stereo drawing of the backbone of PLC. Metal ions are shown as filled circles.



Helices A, B, C, D, F and H₂ form a compact arrangement of mixed parallel and antiparallel helices with a predominantly right-handed twist. Helix D runs diagonally within the helix assembly to lie behind the active site at its C-terminal end (Fig. 2). Although PLC seems to be the first enzyme with an all-helix conformation (citrate synthase contains a 13-residue β -sheet segment⁴¹), several enzymes contain tightly packed helix domains, although there seems to be no common topology (Fig. 2b, I–V).

The N-terminal tryptophan is buried immediately behind the active site and is involved in metal binding (Fig. 3). PLC is probably synthesized as a pro-enzyme with a 14-residue N-terminal extension³ which could block the active site cleft to render the enzyme inactive and could also alter the metal binding. The last two residues are of the C terminus, which lies at the end of helix H₂, disordered on the surface of the molecule. All non-helical stretches form external, 'random coil' loops between the helices (Fig. 2).

The amino-acid sequence of PLC shows no significant similarity to those of phospholipases A₂ (ref. 7). There is, however, some structural similarity with bovine⁸, porcine⁹ and rattlesnake phospholipase A₂ (ref. 10). Superimposition of the antiparallel (residues 39–58 and 89–108) helix pairs in the latter over helices F and H₂ in PLC results in an approximate overlap of the N-terminal helices in phospholipase A₂ over helix A in PLC and a similar location for the N-terminal residue. In this orientation, the β -sheet region of phospholipase A₂ lies over the E–F loop in PLC. The active sites in the two enzymes, however, are well separated when the molecules are thus superimposed.

PLC is inhibited by P_i and by monovalent anions¹¹, including I[−]. The latter ion blocks the entrance to a cleft 5 Å wide, 8 Å deep and bounded on its external surface by the N-terminal loop, the helix B₂–helix C loop, the helix D–helix E loop and the N-terminal end of helix E. The essential metal ions¹² lie at the bottom of this cleft and are liganded to residues from these loops and from helices A, B₂, D and E, thus crosslinking several disparate parts of the protein chain (Fig. 3b). This probably explains the high conformational stability of the holoenzyme relative to the apoenzyme¹³. Furthermore, a 1.9 Å study of P_i-PLC, now approaching completion (*R*-factor = 19%), shows that P_i is closely associated to all three metal ions, with its oxygen atoms replacing two of the coordinated water molecules (Fig. 3c). This confirms the identification of the active site and indicates that the catalytic function of the metal ions is similar to that of the metal ions in alkaline phosphatase from *Escherichia coli*¹⁴, DNA polymerase I (refs 15 and 16) and bovine pancreatic deoxyribonuclease I (ref. 17). There are two distinct groups of residues in the active site: Glu 4, Asp 55, Tyr 56 and Glu 146

form an acidic pocket at one end, whereas Ser 64, Thr 65, Phe 66, Phe 70, Ile 80, Thr 133, Asn 134, Leu 135 and Ser 143 line the remainder. A similar differentiation of active-site residues is discernible in the phospholipases (ref. 18).

Metal bonding and exchange studies^{12,19} have indicated the presence of two tightly bound Zn²⁺ in active PLC. This was apparently confirmed during the search for heavy-atom derivatives by our identification of two sites where Zn²⁺ was replaced with Cd²⁺ (ref. 11). The interpretation of electron density maps in what has now proved to be the N-terminal loop region, however, has always been complicated by the presence of high electron density, similar to that of the other two metal ions, which was closely associated with Trp 1, His 14 and Asp 122, and which could not be explained by amino-acid structure. Contact

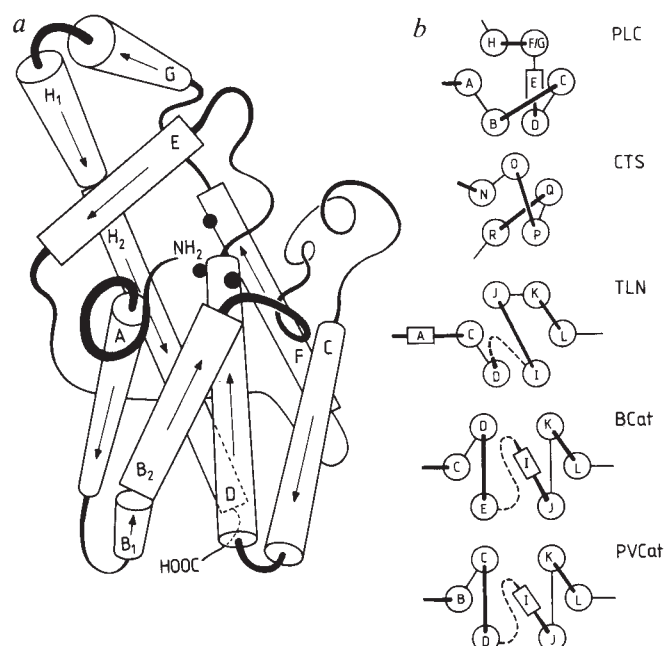


FIG. 2 a, Schematic drawing of the PLC fold with α -helices shown as cylinders and metal ions as filled circles. Overall dimensions are 60 × 40 × 30 Å, 66% of residues fold as α -helix and no β -sheet (predicted²⁸: 30–36% helix, 24–30% sheet). Amino acids in helices: A, 12–28; B₁, 33–44; B₂, 44–55; C, 85–104; D, 105–125; E, 140–153; F, 171–187; G, 192–202; H₁, 206–216; and H₂, 216–243. b, Topology of PLC compared with helix domains in CTS (small domain)⁶, thermolysin (TLN)²⁹, beef catalase (BCat)³⁰ and *Penicillium vitale* catalase (PVCat)³¹. Figures constructed after Levitt and Chothia³².

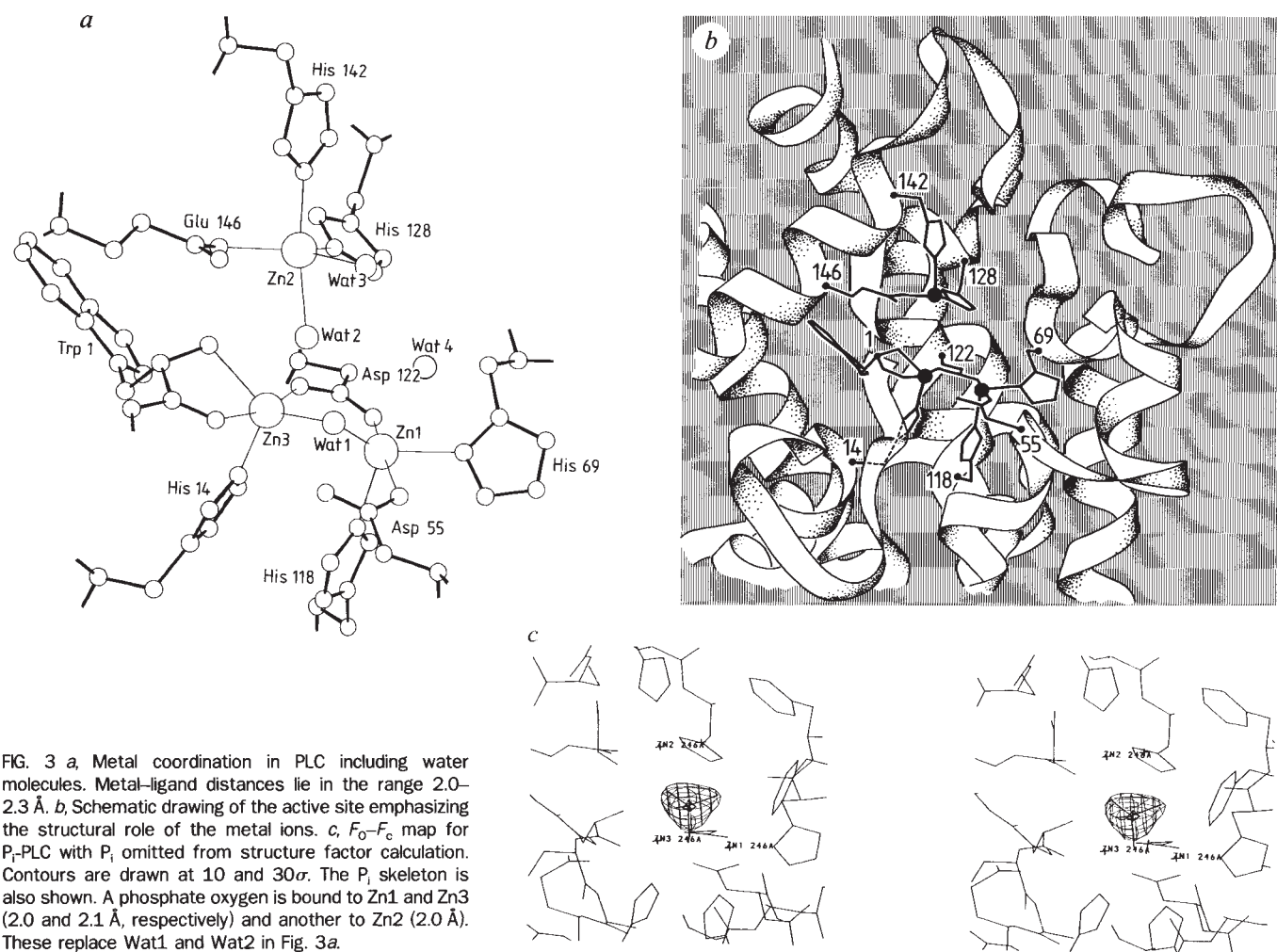


FIG. 3 *a*, Metal coordination in PLC including water molecules. Metal-ligand distances lie in the range 2.0–2.3 Å. *b*, Schematic drawing of the active site emphasizing the structural role of the metal ions. *c*, $F_0 - F_c$ map for P_1 -PLC with P_1 omitted from structure factor calculation. Contours are drawn at 10 and 30σ . The P_1 skeleton is also shown. A phosphate oxygen is bound to Zn1 and Zn3 (2.0 and 2.1 Å, respectively) and another to Zn2 (2.0 Å). These replace Wat1 and Wat2 in Fig. 3*a*.

TABLE 1 Summary of crystallographic data

Derivative	N_h		Resolution limit (Å)							
			14.1	6.45	5.00	4.20	3.65	3.37	3.10	2.80
Cd^{2+} *	2	F	0.68	0.79	0.72	0.67	0.87	0.79	0.75	0.85
		R_c	0.79	0.64	0.75	0.69	0.75	0.76	0.76	0.70
Cd^{2+} †	2	F	1.31	0.96	0.90	0.70	0.64	0.51	0.55	—
		R_c	0.57	0.70	0.61	0.70	0.69	0.73	0.64	—
Pt_6^{2-} *	1	F	0.43	0.61	0.60	0.55	—	—	—	—
		R_c	0.69	0.69	0.71	0.75	—	—	—	—
$PtCl_4^{2-}$ *‡	3	F	1.12	1.08	1.03	0.84	0.72	0.74	0.88	0.84
		R_c	0.65	0.67	0.76	0.74	0.70	0.78	0.68	0.76
I*	6	F	1.42	1.32	1.17	0.97	0.76	0.81	1.02	1.10
		R_c	0.57	0.56	0.56	0.55	0.52	0.53	0.55	0.63
No reflections			406	677	846	989	1,091	1,220	1,314	1,137
Mean figure of merit			0.95	0.92	0.92	0.90	0.84	0.80	0.71	0.58

Crystallization: vapour phase diffusion; 5 mg ml⁻¹ PLC in 35% saturated ammonium sulphate; crystals up to 1.5 mm obtained in 3–4 weeks³³.

Space group: $P4_32_12$; $a = 89.93(3)$ Å, $c = 73.99(4)$ Å; solvent content, 53%; $M_r = 28,585$; 1 molecule per asymmetric unit; data collected on Enraf-Nonius CAD4 diffractometer; native data (1.5 Å) collected on Station 9.6 at Daresbury, U.K.³⁴, $\lambda = 0.88$ Å, 51 film packs, 235,379 reflections merged to 46,061 unique data (99.2% of possible), R_{sym} for $h_{kl} = 0.056$.

Space group ambiguity was resolved by calculation of SIRAS phases to 4 Å using the anomalous differences of the Cd^{2+} derivative measured with Cr-K α radiation in $P4_32_12$ and $P4_32_12$; only the latter gave sensible difference maps for the other derivatives.

Abbreviations: N_h , number of heavy atom sites; F , (r.m.s. heavy-atom structure factor)/(r.m.s. lack of closure); R_c , centric R -factor ($\sum(|F_{PH} - F_P| - |F_H|)/\sum|F_{PH} - F_P|$) where F_{PH} and F_P are the structure factor amplitudes observed for derivative and native respectively, and F_H is the calculated heavy-atom structure factor amplitude).

Refinement: Konnert-Hendrickson with standard restraints³⁵, $R = 15.7\%$ at 1.5 Å for 45,743 reflections (1.9 Å diffractometer data merged with 1.5 Å synchrotron data) with no $\sigma F/F$ cutoff and including 235 water molecules. Deviations (r.m.s.) from ideality are: bond distances, 0.021 Å; angle distances, 0.049 Å; planarity 0.014 Å. Luzatti plot³⁶ corresponds to r.m.s. coordinate error of 0.15 Å. Final refinement and the assignment of water molecules is not yet complete.

* Graphite-monochromated Cu-K α radiation.

† Cr-K α radiation with vanadium β -filter.

‡ Group scattering factors³⁷ used for $PtCl_4^{2-}$.

distance and charge considerations lead us to conclude that this density is due to a third Zn^{2+} (no other metals have been detected in native PLC), which is liganded to the amino and carboxyl groups of Trp 1 to form a five-membered chelate ring similar to that observed in many Zn-amino acid complexes (for example, with aspartic²⁰ and glutamic²¹ acids). The third Zn^{2+} is also coordinated to His 14 and Asp 122.

The resulting constellation of metal ions resembles that in alkaline phosphatase from *E. coli*¹⁴; one of a family of enzymes that have an absolute requirement for Zn^{2+} but can bind other metals with retention of activity²². The Zn-Zn distances in PLC are similar to those reported for Cd-substituted alkaline phosphatase¹⁴. Interestingly, the synthesis of both PLC and alkaline phosphatase are P_i -repressed in *B. cereus* (P. M. Guddal, K. Schulstad, T. Johansen and C.L., unpublished observations) and together represent a P_i retrieval system for the bacterium. The similarity in the active sites in the two enzymes may therefore be more than coincidental (alkaline phosphatase hydrolyses the product from PLC).

Figure 3a shows the environment of the metal ions. Asp 122 forms a carboxylate bridge between Zn1 and Zn3 (Zn-Zn distance 3.3 Å). Two such bridges occur in myohemerythrin (Fe-Fe, 3.23 Å) (ref. 23) and are probably formed by Asp 51 in AP (ref. 14) and between the metal ions in DNA polymerase I (ref. 16). A molecule of water or OH^- forms a second bridge between Zn1 and Zn3; again this is similar to the oxide bridge in myohaemerythrin. The coordination of Zn2 resembles that in carboxypeptidase²⁴, except that only one of the carboxyl oxygens from Glu 146 is bonded to the metal ion. Metal coordination is completed by two further water molecules so that all three metal ions are approximately trigonal bipyramidal. Histidines 14 and 118 are approximately parallel and are oriented in a manner which resembles charge transfer stacking with a ring-ring distance of 3.7 Å.

Atomic absorption analyses of the metal content of PLC (refs 12, 19 and 25) have consistently indicated two metal ions per mol of PLC and the same is true of a solution state EXAFS study²⁶. The metal stoichiometry, however, was calculated from atomic absorption analysis using a relative molecular mass (M_r) of 23,000 obtained from calibrated gel filtration²⁷. Recalculation using the true M_r of 28,520 or 28,585, in the case of three Zn ions³, gives 2.3 Zn ions per mol PLC, indicating that the third metal site is at least partially occupied even in solution. Furthermore, both the analyses and the EXAFS study were carried out on PLC after an ion-exchange^{12,13,19,26} or multi-stage dialysis treatment²⁵ to remove unbound metal ions, a treatment which would also remove loosely bound metal ions. By contrast, crystallization was performed in the presence of $\sim 10 \mu\text{M}$ Zn^{2+} . □

Note added in proof: PLC has extensive homology with the first two-thirds of the recently published sequence of the alpha-toxin (phospholipase C) of *Clostridium perfringens*³⁸⁻⁴⁰.

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ERRATA

Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel

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Nature **337**, 555-557 (1989).

FIGURES 2 and 3 of this letter were inadvertently transposed during the editing process.

Geochemical implications of the formation of the Moon by a single giant impact

H. E. Newsom & S. R. Taylor

Nature **338**, 29-34 (1989).

THE cover for the 2 March issue of *Nature* (shown right), illustrating the Review Article by Newsom and Taylor, was not properly credited. The computation to provide the image was done at Sandia National Laboratories, Albuquerque by Marlan Kipp and H. J. Melosh. Calculations were performed using the new 3-D code CTH on a Cray I XMP with a solid-state hard disk. The full computation required some 60 hours of computational time.

